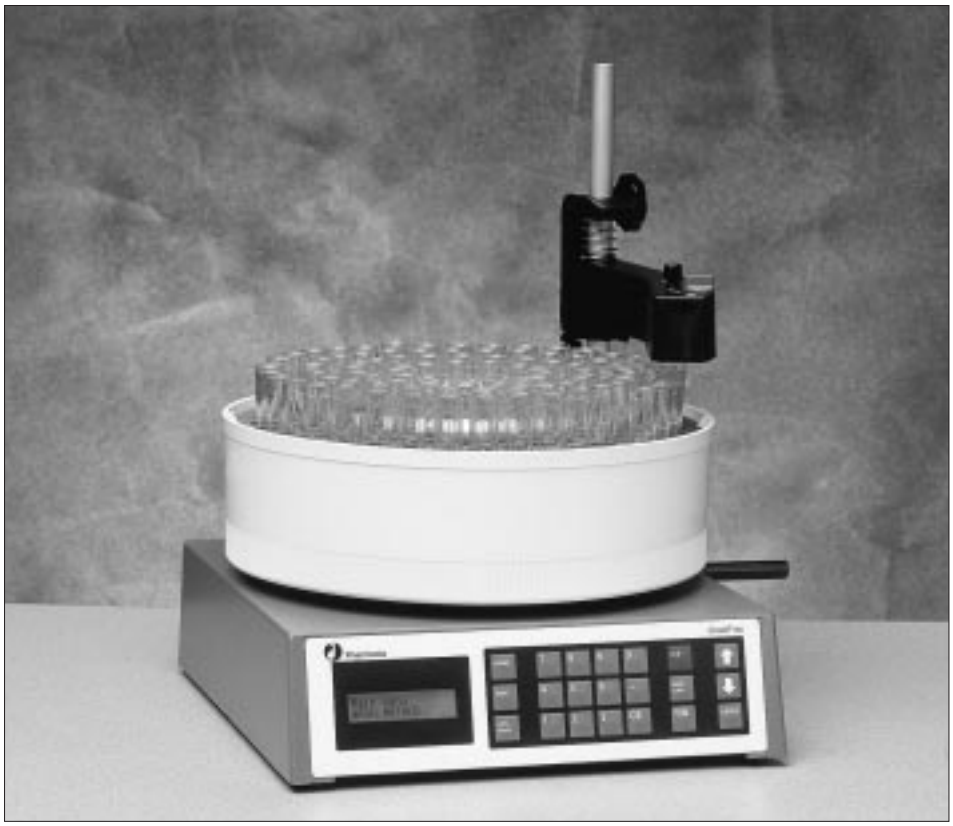
GradiFrac™



User Manual

56-1006-08
Edition AF

Amersham Biosciences

Important user information

Reading this entire manual is recommended for full understanding of the use of this product.

The exclamation mark within an equilateral triangle is intended to alert the user to the presence of important operating and maintenance instructions in the literature accompanying the instrument.

Should you have any comments on this manual, we will be pleased to receive them at:

Amersham Biosciences AB
S-751 82 Uppsala
Sweden

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Contents

1. Introduction 3
2. Description 4
   - 2.1 Front Panel 4
   - 2.2 Rear panel 5
   - 2.3 Power supply 6
   - 2.4 Tube racks 6
   - 2.5 Delivery arm and tube sensor 7
   - 2.6 Solenoid valves 7
   - 2.7 Delivery tubing 8
3. Accessory equipment 9
   - 3.1 System components 9
   - 3.2 Rack 9
   - 3.3 Pumps 9
   - 3.4 Mixer 10
   - 3.5 Solenoid valves 10
   - 3.6 Injection valve 10
   - 3.7 Recorder 10
   - 3.8 Monitor 11
4. Unpacking and assembly 12
   - 4.1 Unpacking 12
   - 4.2 Assembling tube racks 12
   - 4.3 Mounting the bowl 15
   - 4.4 Inserting tubes 16
   - 4.5 Adjusting the delivery arm 16
   - 4.6 Fitting the delivery tubing 16
   - 4.7 Mounting solenoid valves 16
   - 4.8 System mounting 17
   - 4.9 System connection 18
   - 4.10 Tubing connections 20
   - 4.11 Starting the system 21
   - 4.12 Turning the system off 21
5. Setup and programming 22
   - 5.1 GradiFrac Main menu 22
   - 5.2 Setup menu 22
   - 5.3 Calibrating the pump 25

5.4 Program Method menu 26
5.5 Programming a new method....26
5.6 Editing an existing method 29
5.7 Documenting methods....30
5.8 Method examples 30
6. Operation 33
6.1 Manual operation....33
6.2 Method-controlled operation 35
7. Maintenance 37
8. Troubleshooting....38
9. Technical specifications 40
10. Accessories....41
11. Ordering information 42
Appendix: System configuration 44
Index 46

1. Introduction

GradiFrac™ (Code No. 18-1993-01) from Amersham Biosciences AB combines the functions of gradient programmer and programmable fraction collector for standard chromatography applications. It forms the basic unit of GradiFrac System, which includes GradiFrac, Pump P-1, Monitor UV-1, Recorder REC 102, Valve IV-7, Switch Valve PSV-50 and a mounting rack. Gradients are generated using a single pump and a switch valve.

Easy-to-use programming allows automatic gradient formation for pre-programmed separation methods. Up to ten methods can be stored in the instrument memory. Stored methods are retained in memory even when the power is switched off. GradiFrac can also be used in manual control mode, and manual adjustments can be made while a method is running.

GradiFrac can be programmed to collect fractions based on time, volume or drop count. Fractions can also be collected in a peak detection mode when a suitable monitor is connected. Tube racks accommodate tubes with diameter 10-18 mm and length 50-180 mm.

2. Description

2.1 Front Panel

The front panel consists of an alphanumerical LCD display window and a keyboard with membrane keys.

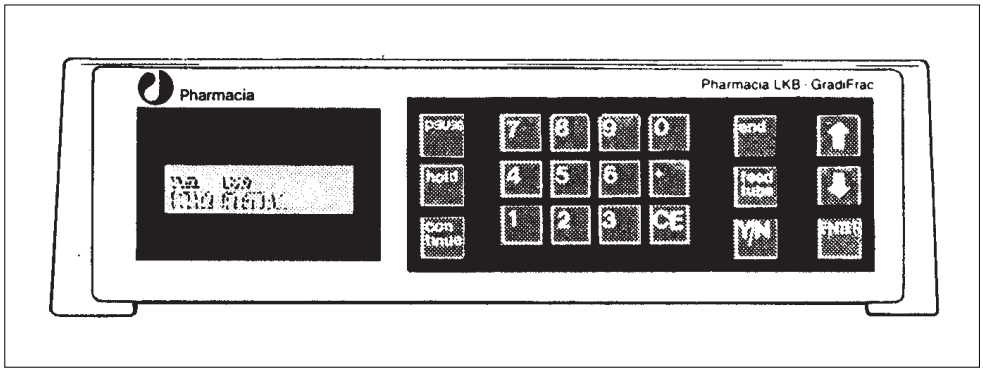


Fig. 1. GradiFrac front panel.

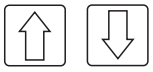
The keyboard is divided into numerical keys and function keys.

2.1.1 Numerical keys

are used for entering values during programming and manual control.

2.1.2 Function keys

are used during both programming and operation. The main functions are briefly summarized below, and are described in more detail as required in the appropriate sections.



Step through the control and programming menus. You can use the arrow keys whenever the display shows an up, down or double arrow.

Enters parameters as shown in the display during programming and manual control.
Acknowledges error messages.

Stops manual operation.
Interrupts method operation before the method is completed. Finishes work in the various menu blocks and returns to the main menu.

Advances the fraction collector one position (except when GradiFrac is paused) The tube feed is delayed according to the Setup value for **Delay UV to Frac** if the flow rate is not zero.

Switches between display of **Y**es and **N**o answers to prompts in manual control and method programming. Pressing **ENTER** accepts the displayed answer.

Pauses all manual or method-controlled operation without ending the method. Pressing continue restarts operation.

hold

Holds method time or volume and holds gradient at the current concentration. All other functions continue uninterrupted. Pressing continue restarts the gradient.

con tinue

Resumes normal operation from the paused or held state.

2.2 Rear panel

The power supply switch and electrical connections for accessories are mounted on the rear panel.

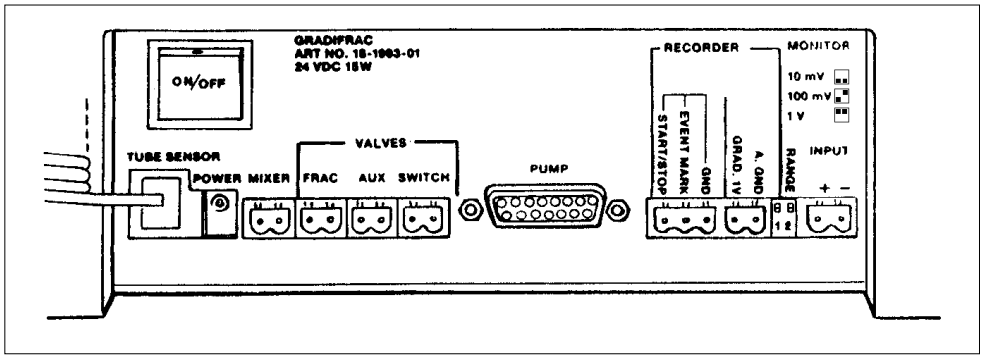


Fig. 2. GradiFrac rear panel.

The rear panel switches and connectors are described briefly below. See Section 4.9 for more details of connecting accessories to GradiFrac.

ON/OFF When the red circle is visible the instrument is off.

Tube sensor Connector for tube sensor and drop counter (mounted on the delivery arm).

Power Connector for 24 V DC power supply. A power conversion unit for use with 100-240 VAC mains is supplied with GradiFrac.

Mixer Connector for supplying power to Amersham Biosciences Mixer 0.6 ml.

Frac Connector for flow diversion valve (PSV-50), which diverts flow between fraction tubes and waste.

Aux Connector for auxiliary valve (PSV-50).

Switch Connector for gradient switch valve (PSV-50).

Pump 15-pin D-SUB connector for control of pump (e.g. Pump P1 or HiLoad Pump P-50).

Recorder

Start/Stop Signal for starting and stopping the recorder chart.

Event mark Event marker signal, activated at fraction collector start and at each tube change.

GND Ground for start/stop and event marker signals.

Grad 1V Signal for recording the gradient (0-1 V corresponding to 0–100% B).

A GND Analogue ground for the gradient signal.

Monitor

Range DIP switches to set the monitor signal range. The switches should be set to correspond to the full-scale output from the monitor (Figure 3).

Input Input connector from the monitor. The monitor signal is used for peak detection.

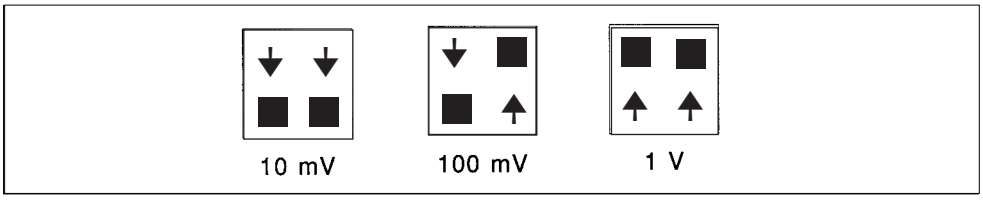


Fig. 3. DIP switch settings for monitor output voltages.

2.3 Power supply

GradiFrac uses a 24 V DC power supply, eliminating the need for fuses or circuit breakers in the instrument. A power conversion unit for use with 100-240 VAC mains is supplied with GradiFrac.

The power conversion unit must be placed in a dry place.

Note: GradiFrac should only be connected to a power supply recommended by Amersham Biosciences.

2.4 Tube racks

Fraction tubes are held in racks which fit into the bowl of GradiFrac. Three standard racks are available:

1. 12 mm rack for 175 tubes, diameter 12 mm.
2. 18 mm rack for 95 tubes, diameter 10-18 mm.
3. 30 mm rack for 40 tubes, diameter 30 mm.

GradiFrac is delivered with the 18 mm rack. The other racks are available as optional accessories.

The 12 mm and 18 mm racks can be assembled to accommodate tube lengths 50-85 mm or 85-180 mm. The 30 mm rack can be assembled to accommodate tube lengths 30-50 mm, 50-85 mm or 85-180 mm. For correct operation, all tubes in a collection sequence must be of the same size. See Section 4.2 for details of tube rack assembly.

Note: In the 18 mm rack, tubes longer than 100 mm should have diameters at least one tenth of the length. Otherwise pressure from the delivery arm can force the tube toward the centre of the rack.

Tubes are arranged in the rack in a counter-clockwise spiral from the outside edge in to the centre. Tube positions are numbered on the tube holders.

Custom tube racks can be made from block styrofoam or by drilling tube holders from plastic plates of the appropriate size. It is important that the angle and distance between tubes are not too great so that tube changes can be made smoothly.

During operation, the bowl and tube rack are turned by the rubber-covered drive sleeve at the side of the GradiFrac base. The position at which the rack stops for each tube is determined by the tube sensor on the delivery arm (see below), so that the rack will not stop in an empty tube position. A safety feature stops collection automatically if rotation of the bowl is obstructed.

2.5 Delivery arm and tube sensor

The delivery arm carries the delivery tubing, tube sensor and drop counter. The cable (carrying both tube sensor and drop counter signals) is connected to the **Tube sensor** contact on the rear panel or GradiFrac.

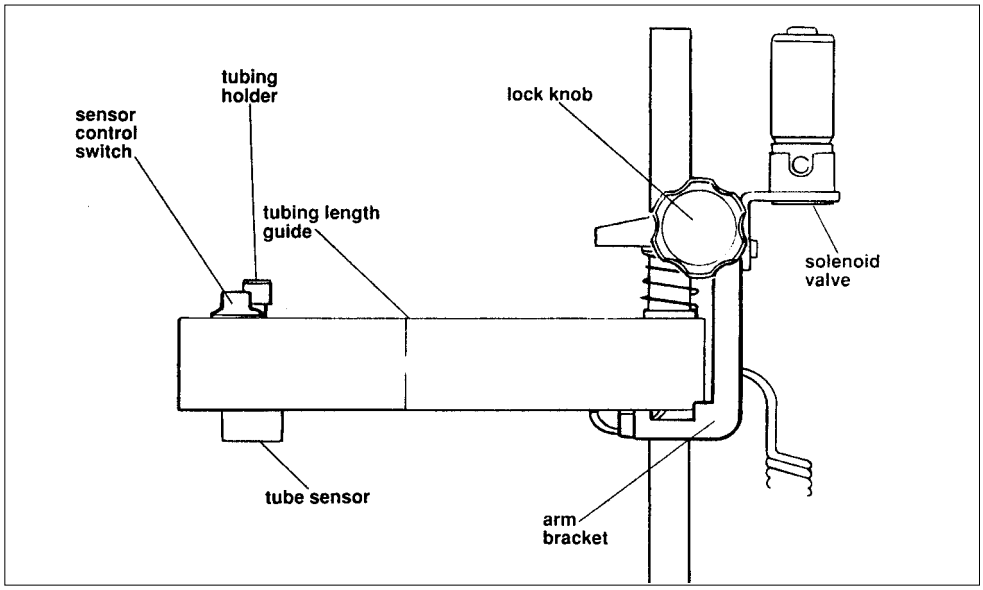


Fig. 4. Delivery arm and tube sensor.

The delivery arm has three stationary positions. At rest it is located over the waste collector in the centre of the bowl. It can also be drawn gently outwards to two positions beyond the radius of the bowl for unhindered access to the tube rack. During operation, the delivery arm is tensioned inwards and rests with the tube sensor against the edge of a tube (where present). The height of the arm can be adjusted with the lock knob on the support rod.

The tube sensor detects the presence of tubes in the tube rack. If a tube is missing, the delivery arm will swing inwards until a tube is detected or the centre position is reached. Note that the spiral tube sequence will be interrupted in such circumstances.

The red sensor control switch at the end of the delivery arm adjusts the tube sensor for large or small diameter tubes. Turn the switch to the smaller marking for tubes in the 12 mm rack, and to the larger marking for tubes in the 18 and 30 mm racks.

The delivery tubing is inserted into the tubing holder. A tubing length guide is provided midway along the upper side of the delivery arm to help in positioning the end of the delivery tubing correctly with respect to the drop counter. This guide is a small hole with a white ring. See Section 4.6 for a description of how to fit the delivery tubing correctly.

2.6 Solenoid valves

The supplied solenoid valves PSV-50 may be connected to the ports marked **Frac** and **Switch** on the rear panel of GradiFrac, with the following functions:

Frac Diverts flow to waste when fractions are not being collected (fraction size is set to 0).

Switch Switch valve for gradient formation.

The port marked **Aux** is for an optional third solenoid valve, e.g. for three-buffer systems and for automatic application of large sample volumes.

Solenoid valve PSV-50 has three outlets:

IN

NO Normally open

NC Normally closed

See Figures 13 and 14 for tubing connections to the valves.

2.7 Delivery tubing

0.8 mm i.d. tubing is recommended and is supplied with GradiFrac. Tubing connector/M6 (Code No. 19-1993-50) is used for connection.

3. Accessory equipment

3.1 System components

Recommended components for use together with GradiFrac are listed below. Some typical system arrangements are illustrated in the appendix.

Component	Recommended models
Rack	GradiFrac System Rack
Pumps	Peristaltic Pump P-1 HiLoad™ Pump P-50
Solenoid valves	PSV-50 for gradient formation PSV-50 for flow diversion PSV-50 for sample application or three-buffer systems (optional)
Manual valves	IV-7
Mixer	Mixer 0.6 ml
Monitor	UV-1
Recorder	REC 102
Columns	Prepacked HiLoad™ and HiTrap™ columns (see Chapter 10)

3.2 Rack

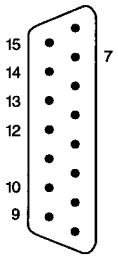
A rack (Code No. 18-1993-05) is available for convenient mounting of system components.

3.3 Pumps

Recommended pumps for use with GradiFrac are Pump P-1 for flow rates 0-8.3 ml/min and HiLoad™ Pump P-50 for flow rates up to 50 ml/min. Other pumps may also be used provided that the pump speed is frequency-controlled.

GradiFrac controls pump speed by a pulsed output signal with frequency range 0-4 kHz. The programmable flow rate range is 0–99.9 ml/min. The pump calibration constant can be entered directly if known, or the pump can be calibrated by collecting a measured volume. See Section 5.3 for details of pump calibration.

Connection to GradiFrac: 15 pin D-SUB.
Recommended cable: Communication cable (Part No. 19-6005-02).



Pump connector pin assignments:

Pin no	Function
(1-6)	(Not used in GradiFrac)
7	0 V (ground)
(8)	(Not used in GradiFrac)
9	Pump busy
10	Pump operable
(11)	(Not used in GradiFrac)
12	Pump control
13	Frequency from pump (for calibration)
14	Frequency to pump (pump speed)
15	0 V (ground)

As long as GradiFrac is switched on, the pump is controlled entirely from GradiFrac. To use the controls on the pump itself, switch off the power to GradiFrac or disconnect the pump communication cable.

3.4 Mixer

Amersham Biosciences Mixer 0.6 ml is suitable for most applications using GradiFrac. For high flow rates (above 8 ml/min), Mixer 6.0 ml is recommended.

Mixer 0.6 ml is connected to the Mixer port on the rear panel of GradiFrac, which supplies power to the mixer (24 V AC, 50 Hz). The mixer operates as long as GradiFrac is turned on.

Mixer 6.0 ml cannot be supplied with power from GradiFrac and requires a separate power source.

3.5 Solenoid valves

Solenoid valve PSV-50 should be used with GradiFrac. PSV-50 may be connected to the ports marked Frac, Switch and Aux on the rear panel of GradiFrac, with the following functions:

Frac Diverts flow to waste when fractions are not being collected (fraction size is set to 0).

Switch Switch valve for gradient formation.

Aux Optional third switch valve, e.g. for three-buffer systems and for automatic application of large sample volumes.

3.6 Injection valve

Injection Valve (IV-7) is recommended for sample application. If desired, a system may be configured with the auxiliary switch valve as a sample application valve (see Appendix).

3.7 Recorder

Recorders such as Amersham Biosciences REC 101 and REC 102 as well as other recorders are suitable for use with GradiFrac.

The recorder is connected to the port(s) marked **Recorder** on the rear panel of GradiFrac. Use the Recorder Cable packed in the GradiFrac box (Code No. 56-1136-67). (The ordering number for the Recorder Cable is 18-1107-88.) The port connections have the following functions:

Start/Stop Start and stop the recorder chart. The chart drive will operate as long as the flow rate is not zero.

Event Event marker signal, activated at fraction collector start and markat each tube change. The signal is a short (50 ms) pulse.

GND Ground for start/stop and event marker signals.

For two-channel recorders, GradiFrac supplies a signal corresponding to the buffer gradient:

Grad 1V Signal for recording the gradient (0–1 V corresponding to 0–100% B).

A GND Analogue ground for the gradient signal.

3.8 Monitor

Monitor UV-1 is recommended for use with GradiFrac. The output from the monitor is connected to the **Monitor +, –** ports on the rear panel of GradiFrac to supply a signal for peak detection functions. Recommended cable: Signal cable (Part No. 19-6006-01).

The **Range** switches on the rear panel of GradiFrac should be set to the full-scale output from the monitor (10 mV for Monitor UV-1).

The output from the monitor should be connected separately to the recorder and to GradiFrac. There is no output from GradiFrac for the monitor signal. An adaptor cable for connecting UV-1 to both GradiFrac and a recorder is supplied with GradiFrac.

4. Unpacking and assembly

4.1 Unpacking

Unpack the GradiFrac carefully and check the contents of the carton against the included packing list. Inspect each item for damage that may have occurred during transit. Save the packing material and carton for later use.

4.2 Assembling tube racks

Tube racks are assembled in the GradiFrac bowl from some or all of tube holder, tube guide and tube support (Figure 5). Details of assembly differ according to the length and diameter of the tubes.

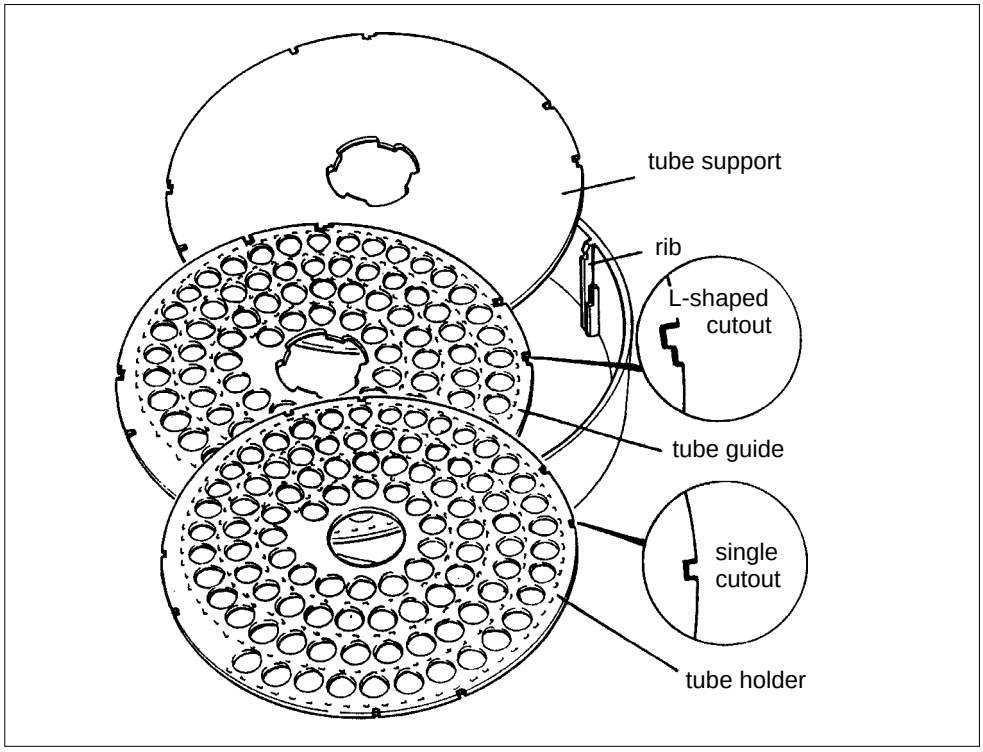


Fig. 5. Tube rack components.

Components of the tube rack are positioned in the bowl by engaging the cutouts (either single or L-shaped) on the edge of the component with the ribs on the inside of the bowl. This ensures that the different components are correctly aligned with each other.

4.2.1 Tube racks 12 mm and 18 mm.

Tube lengths 50-85 mm

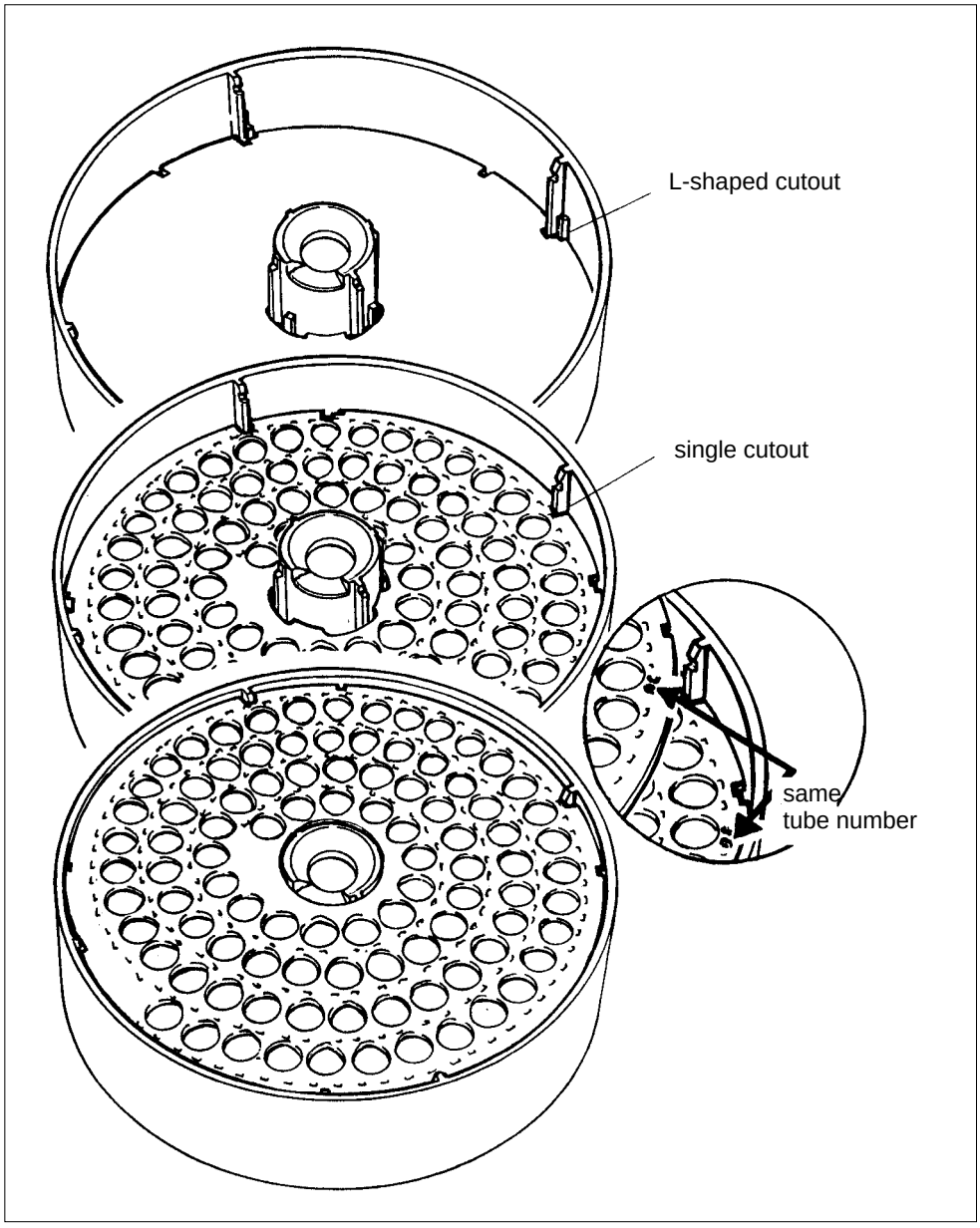


Fig. 6. Assembly of 12 mm and 18 mm tube racks for 50-85 mm tubes.

Fit the tube support, using the L-shaped cutouts. The support should be seated in the lower position in the bowl with the circular marks on the underside.

Fit the tube guide in the bowl with the tube numbers facing up, using the single cutouts. The guide should come to rest approximately 1 cm above the tube support.

Fit the tube holder in the bowl with the tube numbers facing up, using the single cutouts, ensuring that tube position 1 is directly above tube position 1 in the tube guide. The tube holder is held in position by the indentations in the ribs of the bowl. Check that the tube holder is level and firmly seated before filling it with tubes.

Tube lengths 85-180 mm

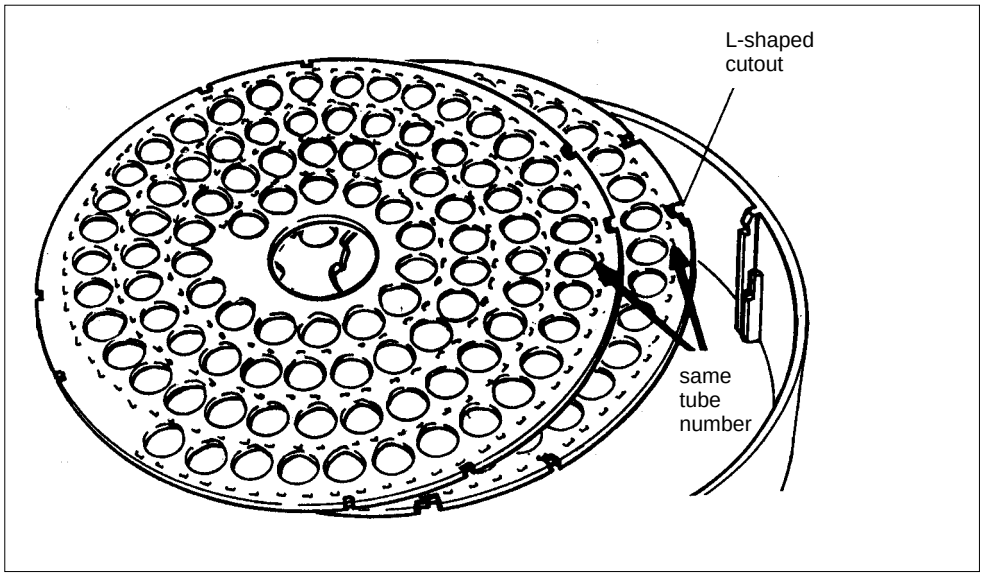


Fig. 7. Assembly of 12 mm and 18 mm tube racks for 85-180 mm tubes.

Do not use the tube support.

Fit the tube guide in the bowl with the tube numbers facing up, using the L-shaped cutouts. The guide should be seated in the lower position in the bowl.

Fit the tube holder in the bowl with the tube numbers facing up, using the single cutouts, ensuring that tube position 1 is directly above tube position 1 in the tube guide. The tube holder is held in position by the indentations in the ribs of the bowl. Check that the tube holder is level and firmly seated before filling it with tubes.

4.2.2 Tube rack 30 mm.

Tube lengths 30-50 mm

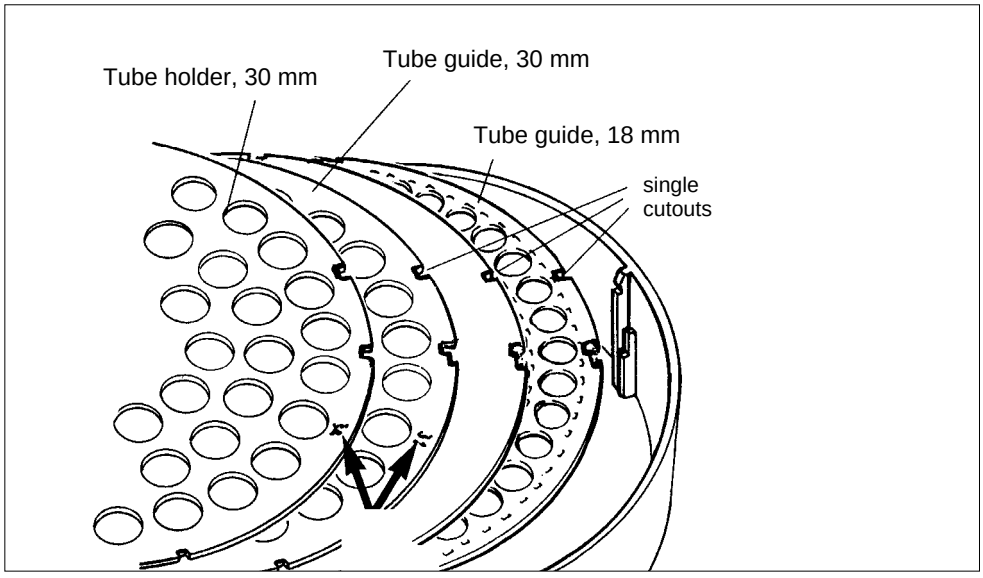


Fig. 8. Assembly of 30 mm tube racks for 30-50 mm tubes.

Fit the tube guide from the 12 or 18 mm rack, using the single cutouts. Fit the tube support on top of the tube guide to give the extra height necessary when using short tubes.

Fit the 30 mm tube guide, using the single cutouts.

Fit the tube holder in the top of the bowl with tube position 1 directly above tube position 1 in the tube guide.

Tube lengths 50-85 mm
Fit the tube support using the L-shaped cutouts. The support should be seated in the lower position.

Fit the tube guide using the single cutouts.

Fit the tube holder in the top of the bowl with tube position 1 directly above position 1 in the tube guide.

Tube lengths 85-180 mm
Do not use the tube support.

Fit the tube guide in the bowl with the tube numbers facing up, using the L-shaped cutouts. The guide should be seated in the lower position in the bowl.

Fit the tube holder in the bowl with the tube numbers facing up, ensuring that tube position 1 is directly above tube position 1 in the tube guide. The tube holder is held in position by the indentations in the ribs of the bowl. Check that the tube holder is level and firmly seated before filling it with tubes.

4.3 Mounting the bowl

Move the delivery arm out to the second stop. Place the bowl containing the rack over the central spindle. Pull the spring-loaded drive sleeve away from the bowl so that the bowl drops into position.

The bowl should come to rest with a gap of approximately 1 cm between the bottom of the bowl and the GradiFrac base.

4.4 Inserting tubes

Insert sufficient collection tubes into the rack beginning at position 1. Ensure that the tubes are pushed down as far as possible. All tubes in one collection sequence must be of the same length and diameter and there should be no empty spaces.

4.5 Adjusting the delivery arm

1. Move the arm by lifting it a little so that the tube sensor comes to rest against a tube in the outer row.
2. Loosen the lock knob on the column.
3. Adjust the height of the arm so that the tube sensor is about 5 mm below the top of the tubes and tighten the lock knob.
4. Rotate the rack anti-clockwise by hand until the sensor comes to rest against the tube in position 1. Ensure that the red sensor switch is correctly set for the tube diameter (to the smaller marker for 12 mm racks and to the larger marker for 18 and 30 mm racks).

4.6 Fitting the delivery tubing

1. Cut the end of the delivery tubing cleanly at 90°.
2. Lift out the tubing holder from the delivery arm and loosen the nut.
3. Fit the delivery tubing through the tubing holder.
4. Place the tubing holder over the length guide hole. Push the tubing through the holder until it reaches the bottom of the length guide, then tighten the nut on the tubing holder. This ensures that drops are formed in the correct position with respect to the drop counter.
5. Fit the tubing holder into the delivery arm.

4.7 Mounting solenoid valves

Screw the valve holder to the back of the delivery arm using the screws provided. Attach the flow diversion valve PSV-50 to the valve holder (Figure 9).

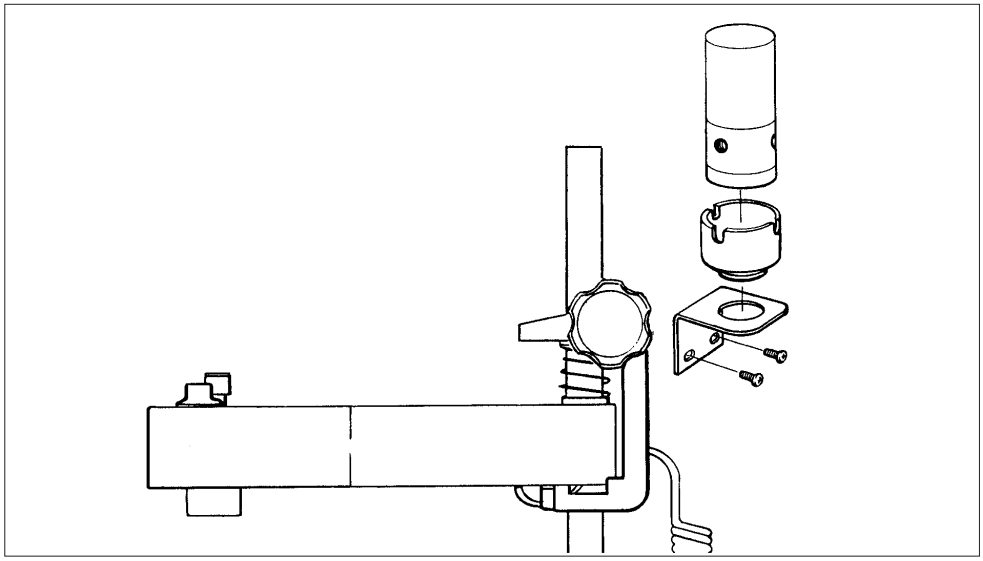


Fig. 9. Mounting the flow diversion valve.

4.8 System mounting

Sections 4.8-4.10 describe assembly of GradiFrac System. System components are listed in Section 3.1. A separate System Installation Guide is available.

1. Prepare GradiFrac as described in Sections 4.2-4.7.
2. Attach the solenoid valve for gradient formation to the valve bracket. If an auxiliary valve is used, attach this to the same valve bracket alongside the gradient valve (Figure 10).

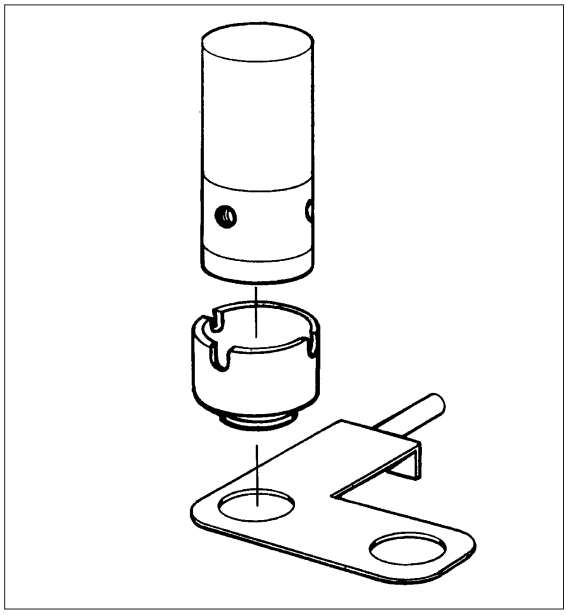


Fig.10. Fitting the gradient valve to the valve bracket. The auxiliary valve can be fitted to the same bracket.

3. Assemble the rack as described in the instructions provided.
4. Prepare the other components for use as described in the respective User Manuals.
5. Place the system components in the rack as shown in Figure 11.

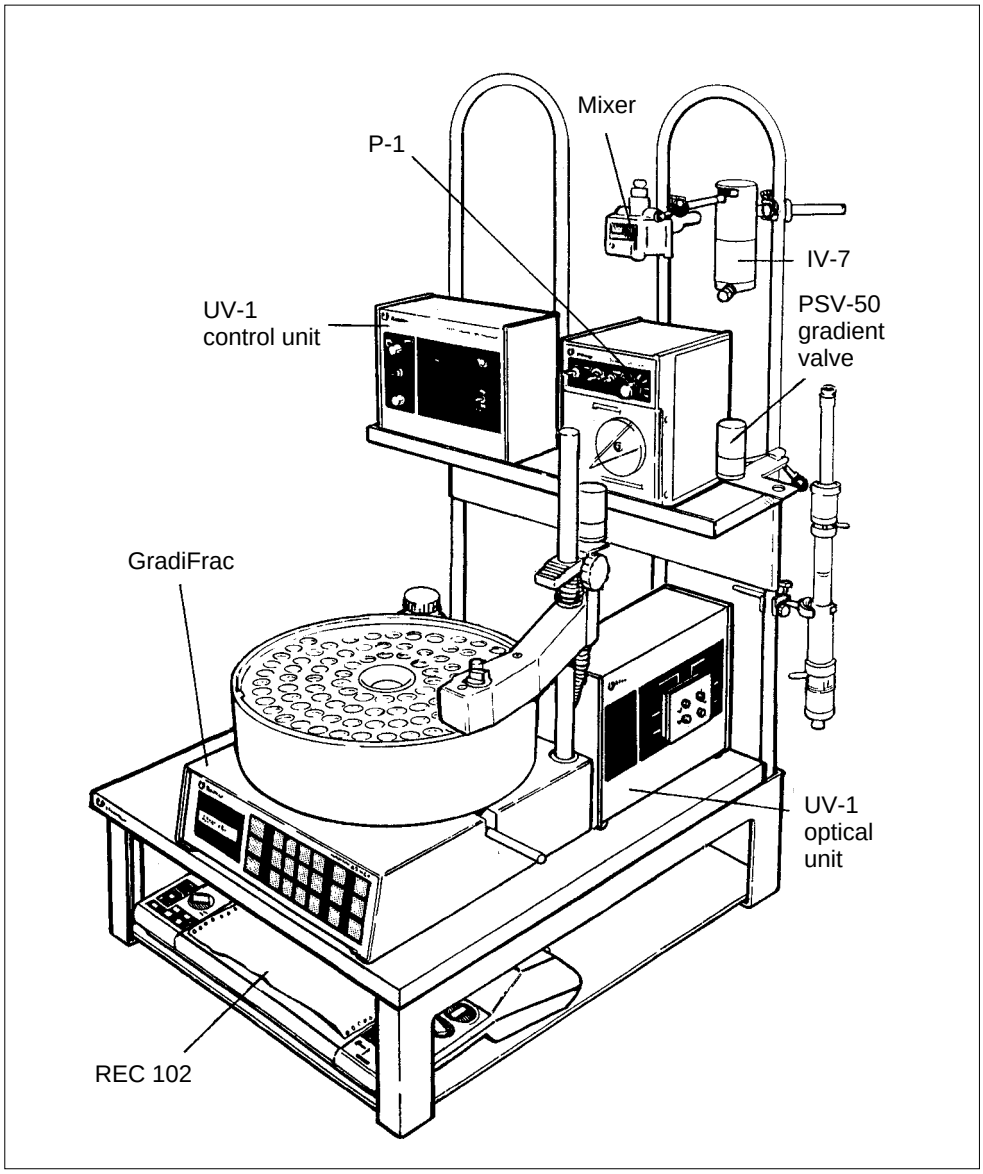


Fig. 11. Placing the system components in the rack.

4.9 System connection

Connect components electrically to GradiFrac as described below (Figure 12).

Use a screwdriver to fit the green connectors supplied with GradiFrac to the valve, recorder and monitor cables that are to be connected to GradiFrac rear panel. Valve cables have no polarity and may be connected either way round. Connect the monitor cable with the polarity shown on GradiFrac rear panel and recorder cables as shown in Figure 12.

All sockets on the rear panel must have their respective green connectors fully inserted to avoid the risk of electrostatic discharge (ESD).

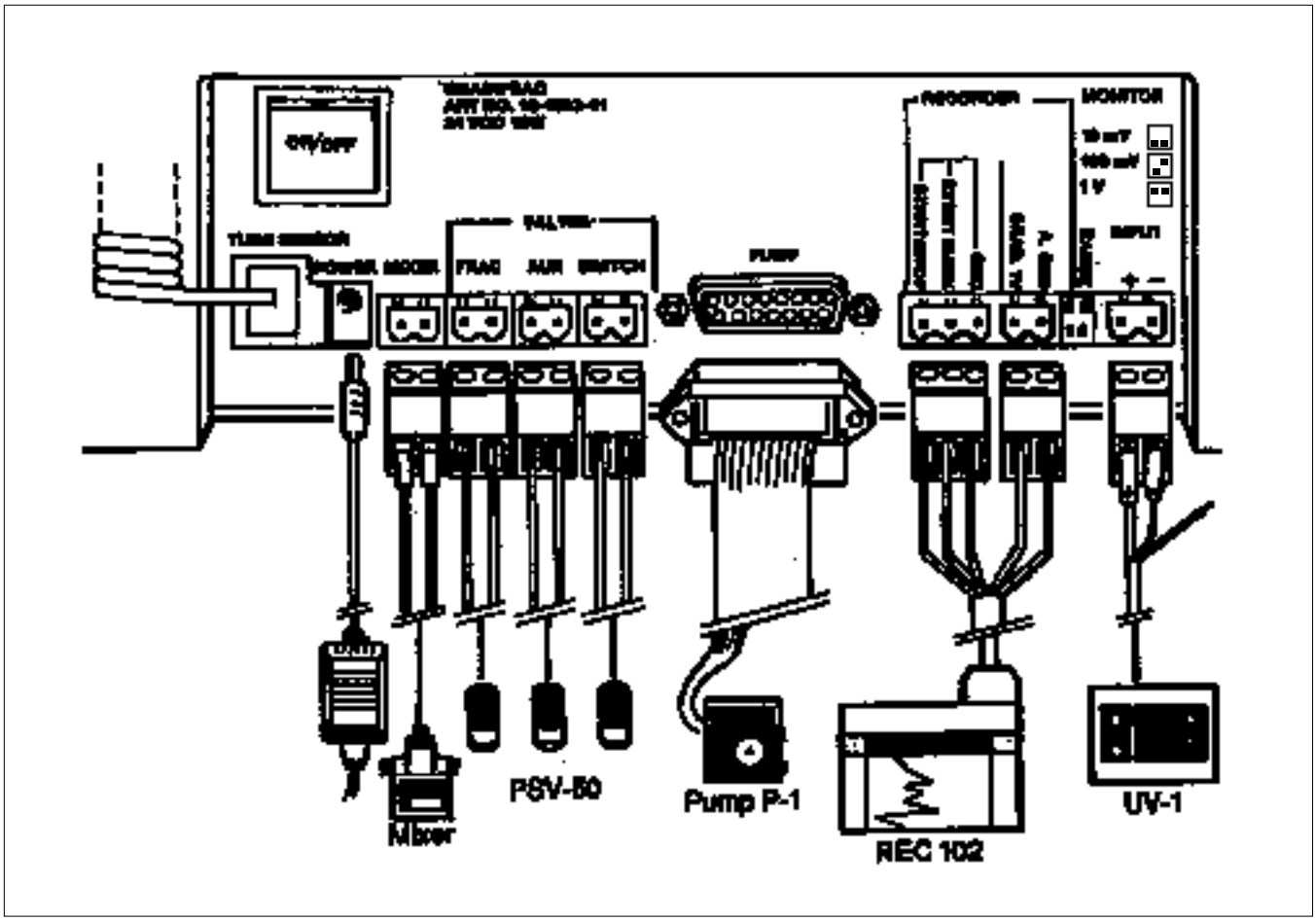


Fig. 12. Electrical connections.

4.9.1 Tube sensor

Connect the tube sensor cable from the delivery arm to the **Tube sensor** contact on the rear panel of GradiFrac. (This cable may already be connected on delivery).

4.9.2 Pump

Connect the **Remote** contact on the pump to the **Pump** contact on the rear panel of GradiFrac.

4.9.3 Valves

Connect the flow diversion valve to the **Frac** port on the rear panel of GradiFrac.

Connect the gradient valve to the **Switch** port on the rear panel of GradiFrac.

Connect the auxiliary valve, if used, to the **Aux** port on the rear panel of GradiFrac.

4.9.4 Mixer

Connect Mixer 0.6 ml to the **Mixer** port on the rear panel of GradiFrac.

4.9.5 Recorder

Connect the recorder to the three-pin port marked **Recorder (Start/stop, event mark, GND)** and the two-pin port marked **Recorder (GRAD 1V, A GND)** on the rear panel of GradiFrac, using the special Recorder Cable packed in the GradiFrac box (Code No. 56-1136-67). (The ordering number for the Recorder Cable is 18-1107-88.)

Connect the 15-pin D-SUB connector to the port on the rear panel of the recorder, and the red and black pin connectors to channel 2 (red) on the top of the recorder.

Set the red switch on the recorder (voltage selector for channel 2) to 1 V.

Note: Chart speed must be set locally on the recorder. The **int/ext** button on REC 102 should be in position i**nt**. The **Rec on/off** button should be in position **on**.

4.9.6 Monitor

Set the DIP switch on the rear panel of GradiFrac (see Section 2.2) to correspond to the full-scale output from the monitor (10 mV for Monitor UV-1).

Use the Y-cable supplied with GradiFrac to connect the monitor output to both GradiFrac and recorder. Connect the monitor output to the **Monitor** port on GradiFrac, taking care to match the **+** and **–** poles. Connect the monitor output separately to channel 1 input on the recorder.

Set the blue switch on the recorder (voltage selector for channel 1) to correspond to the full-scale output from the monitor (10 mV for Monitor UV-1).

4.9.7 Power supply

Connect the 24 V DC outletfrom the power conversion unit to the **Power** socket on the rear panel of GradiFrac. Connect the power conversion unit to the mains supply. The conversion unit adjusts automatically to mains voltages in the range 100-240 V AC, 50-60 Hz.

4.10 Tubing connections

Connect tubing as shown in Figures 13 and 14. Make sure that the tubing connections to the solenoid valves are made to the correct valve outlets as shown in Figure 14.

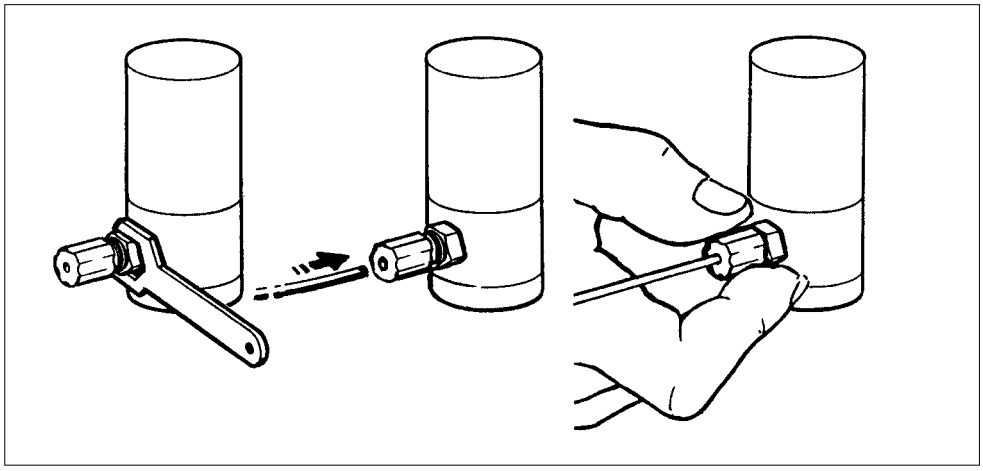


Fig. 13. Connecting tubing to PSV-50.

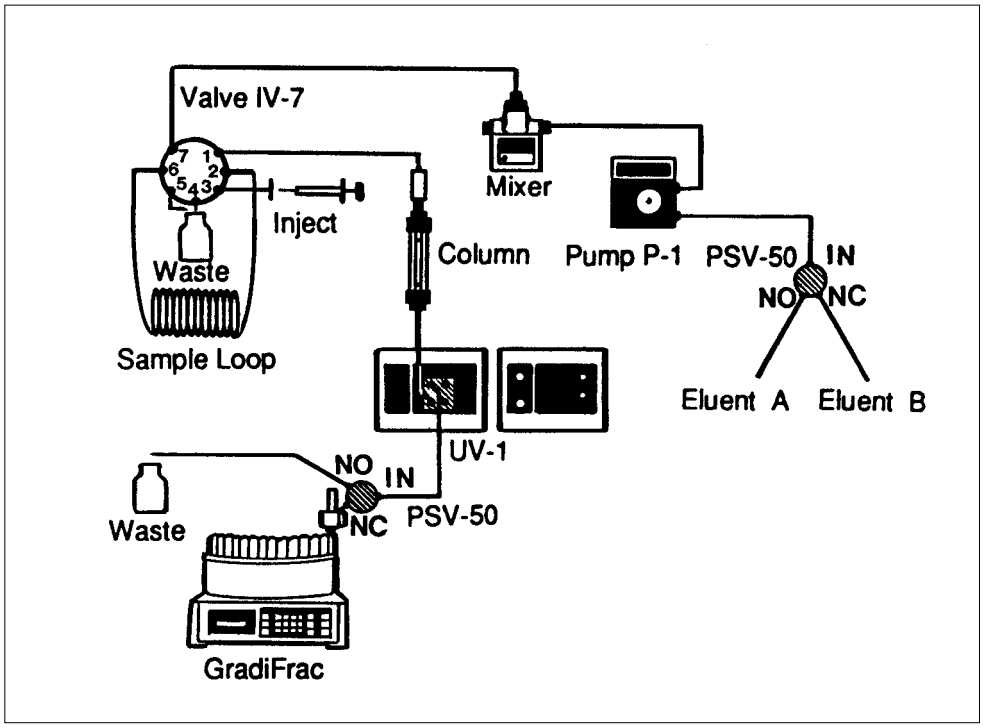


Fig. 14. System tubing connections.

4.11 Starting the system

Turn on the power to GradiFrac first, then to the other components in the system.

4.12 Turning the system off

Turn off the power to the pump and recorder first. If GradiFrac is turned off while power is still supplied to pump or recorder, these instruments will start to run according to the settings on the instruments themselves. This can be particularly important for the pump, since liquid or air may otherwise inadvertently be pumped through the system.

5. Setup and programming

5.1 GradiFrac Main menu

The GradiFrac main menu is divided into four blocks:

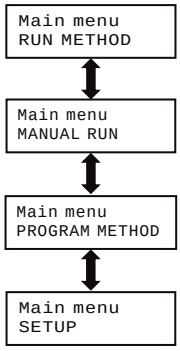


Fig. 15. GradiFrac main menu.

When GradiFrac is first switched on, the **Run method** option is displayed. Press the arrow keys to move between menu options.

5.2 Setup menu

The Setup menu block sets general operation parameters. Setup should be performed when GradiFrac is first installed and whenever parameters need to be changed.

Current setup parameters apply during manual operation and method programming. During method-controlled operation, the **method base** (time/volume) and **fractionation base** (time/volume/drop) which were current at the time of programming apply. All other Setup parameters during method-controlled operation are those which were last entered.

Note that the method base and fractionation base are defined by those used in the most recently accessed method (either in Method Run or in Program method), unless they are actively reset in Setup.

Switch on GradiFrac. After a short interval, the display shows:

Main Menu
Run Method

Press the ⇩ key three times until the display shows

Main Menu
Setup

Press **ENTER** to enter the setup block.

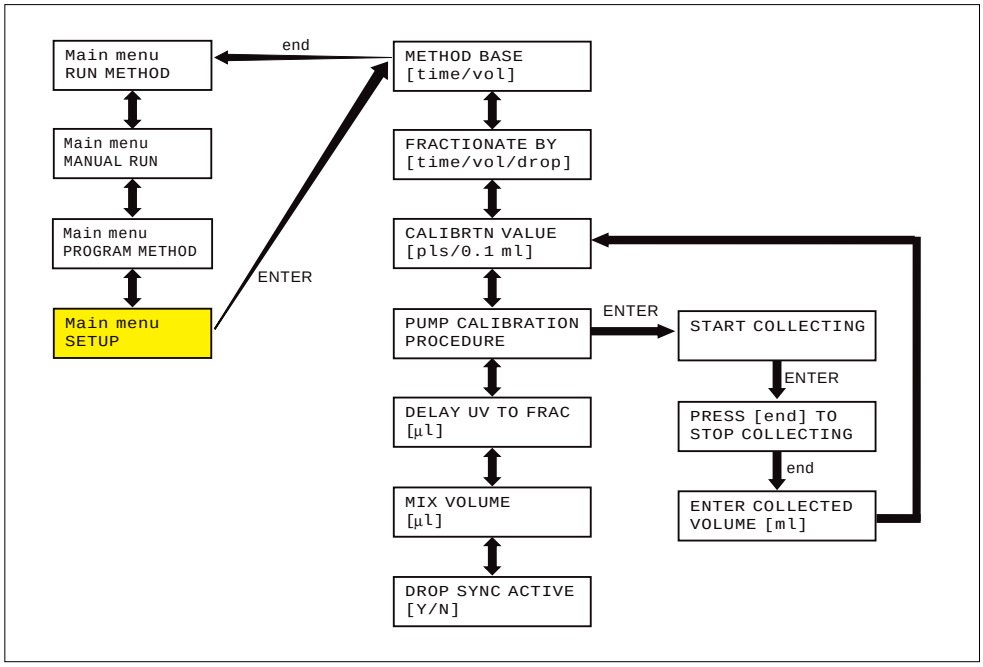


Fig. 16. The Setup menu block.

5.2.1 Setting the method base

Set the method base with **Method base** (Figure 16).

Choose time (min) or volume (ml). The chosen base will apply for all subsequent programming of new methods. Previously stored methods however retain the method base in which they were programmed.

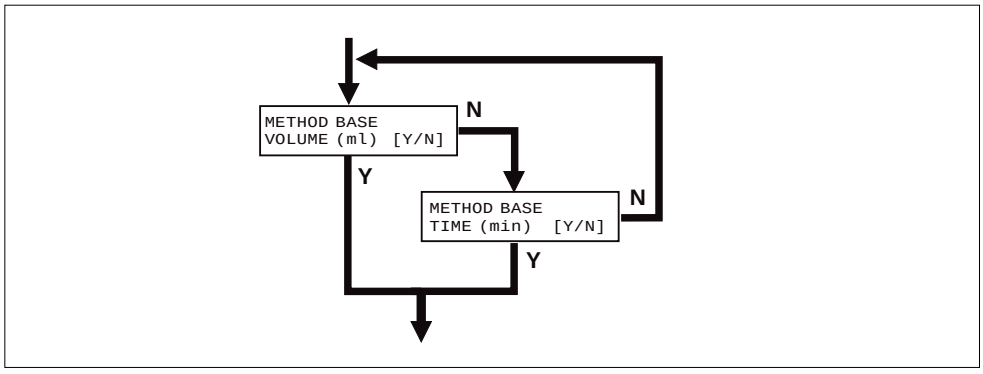


Fig. 17. Setting the method base.

5.2.2 Setting the fractionation base

Set the fractionation base with **Fractionate by** (Figure 16).

Choose time (min), volume (ml) or drops. The chosen base will apply for manual operation and for all subsequent programming of new methods. Previously stored methods however retain the fractionation base in which they were programmed.

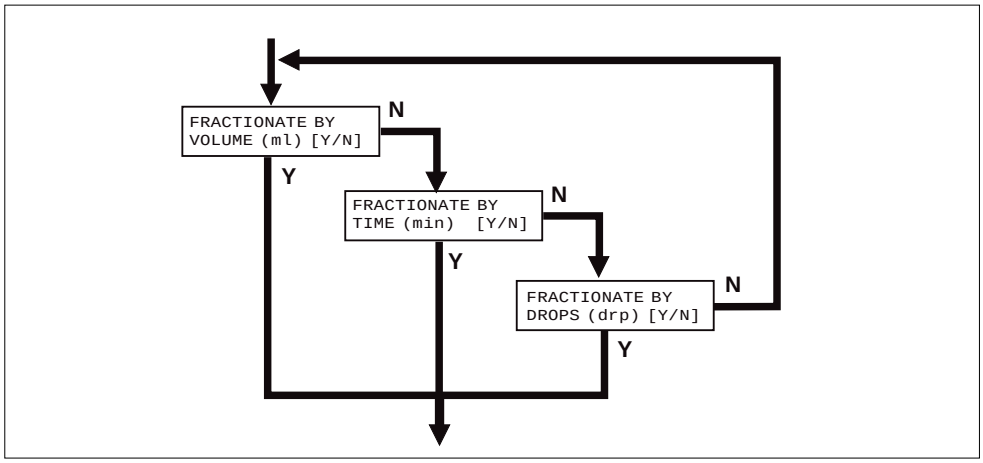


Fig. 18. Setting the fractionation base.

5.2.3 Setting the pump calibration factor

Set the pump calibration factor with **Calibrtn value** (Figure 16).

The pump calibration factor is the number of pulses required to deliver 0.1 ml from the pump. Enter the value directly if known and press **ENTER**. If the calibration factor is unknown, see Section 5.3.

Note that the calibration factor for Pump P-1 depends on the tubing diameter. You are advised always to perform pump calibration whenever the tubing is changed.

The pump calibration factor will apply for all subsequent manual and method-controlled operation until a new value is set.

5.2.4 Setting the monitor-to-collector delay

Set the delay volume between the UV monitor and the fraction collector with **Delay UV to Frac** (Figure 16).

Enter the volume of liquid (in µl) in the delivery tubing between the UV monitor flow cell and the very end of the tubing. This will ensure that event marks on the UV absorbance trace on the recorder are synchronized with tube changes. Calculate the delay volume from the length of tubing. The volume of 0.8 mm i.d. tubing is 50 µl/10 cm.

The delay volume will apply for all subsequent manual and method-controlled operation until a new value is set.

5.2.5 Setting the mixing volume

Set the mixing volume with **Mix volume** (Figure 16).

The mix volume is the total volume (µl) of liquid (buffer A + buffer B) which passes through the gradient valve during one cycle of operation. The value for the mix volume together with the flow rate thus determines the switching frequency of the valve. If the mix volume is too low, the switching frequency may be too high (the minimum cycle time for valve PSV-50 is 30 ms). If the mix volume is too high, the gradient will be inaccurate at low flow rates. The mix volume for Pump P-1 is calculated according to the table below:

Pump tubing i.d. (mm)	Flow rate range (ml/min)	Mixer (ml)	Mix volume (µl)	
1.0	0–1	0.6	Mix volume =	$\frac{54\,000}{\text{pump calibration factor}}$
2.1	1–4	0.6	Mix volume =	$\frac{18\,000}{\text{pump calibration factor}}$
3.1	3–5	0.6	Mix volume =	$\frac{8\,800}{\text{pump calibration factor}}$
3.1	5–8	6.0	Mix volume =	$\frac{18\,000}{\text{pump calibration factor}}$

The following values are recommended for HiLoad Pump P-50:

Flow rate (ml/min)	Mixer (ml)	Mix volume (µl)
0-5	0.6	60
>5	6.0	600

Note that the mix volume is not the same as the volume of the mixer.

The mix volume will apply for all subsequent manual and method-controlled operation until a new value is set.

5.2.6 Setting drop synchronization

Set drop synchronization with **Drop sync active** (Figure 16).

If drop synchronization is active, tube changes will only occur directly after a drop is registered by the drop counter, to minimize spillage between tubes. Drop synchronization operates in all fraction collection modes (time, volume and drop).

Drop synchronization is only possible at flow rates below 3 ml/min.

The drop synchronization setting will apply for all subsequent manual and method-controlled operation until a new value is set.

5.3 Calibrating the pump

To calibrate the pump, move to **Pump calibration procedure** in the Setup menu (Figure 16) and press **ENTER**. Follow the steps below:

1. Set the speed on the pump to a value close to the flow rate which will be used subsequently. For Pump P-1, use the flow rate guide supplied with the pump.
2. Make sure there are no air bubbles in the pump or delivery tubing.
3. Enter the setup block in the GradiFrac main menu.
4. Press ⇩ until the display shows **Pump calibration procedure**, then press **ENTER** to enter the calibration process. The display will show **Start collecting**.
5. Place a suitable measuring vessel under the outlet tubing and press **ENTER**. The pump will start to operate and the display will show **Stop collecting**.
6. Collect a volume large enough for accurate measurement, then press **end** to stop collecting. The pump will stop and the display will show **Enter collected volume**.
7. Measure the collected volume, enter the value (ml) and press **ENTER**. GradiFrac will calculate the calibration factor, and the display will show the calculated value.

8. Press **end** to return to the main menu or ⇩ to continue with the setup menu.

The calibration factor for HiLoad Pump P-50 is 234 pulses/0.1 ml. Enter this factor directly in the Setup menu if Pump P-50 is used. Pump calibration should only be necessary for Pump P-1 and other pumps with unknown calibration factors.

5.4 Program Method menu

GradiFrac can store up to 10 methods of 52 breakpoints each.

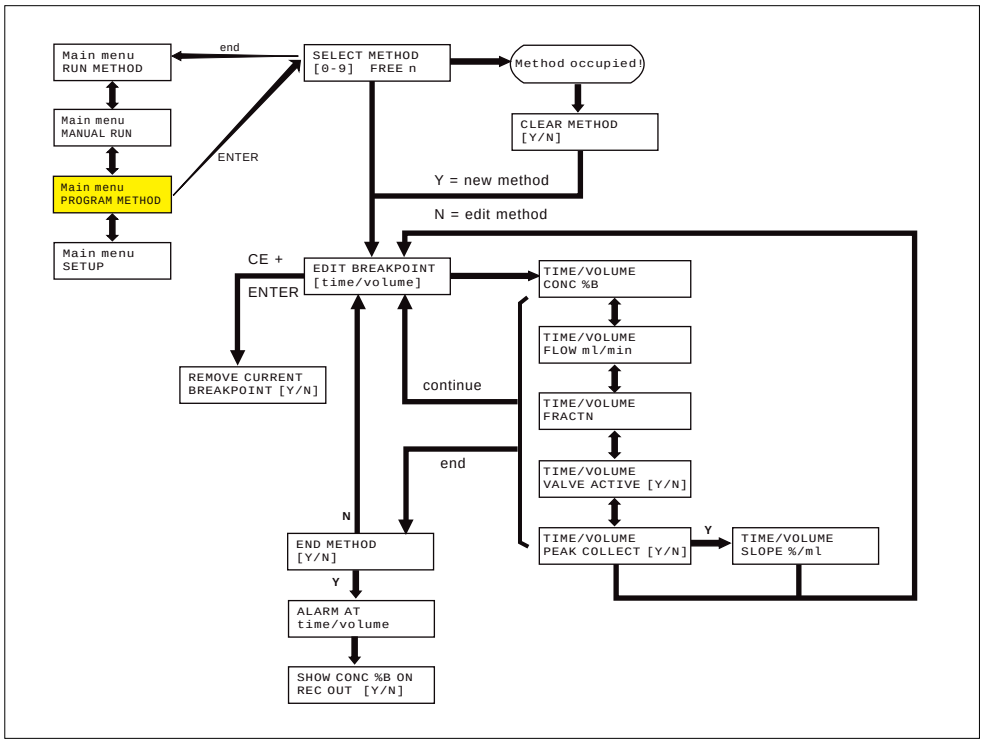


Fig. 19. The Program method control block.

Each holds settings for all method parameters. During programming, the default settings for a new breakpoint are taken from the previous existing breakpoint in the method.

5.5 Programming a new method

5.5.1 Selecting a method number

Choose Program Method in the main menu (Figure 15) and press **Enter**.

Enter the number of the method to program. GradiFrac shows the number of the lowest empty method in the window, and suggests the most recently used number as default.

If the method number is occupied, GradiFrac responds with the message

Method occupied!

To choose a new method number, press **end** to return to the main menu.

To clear the existing method, press **ENTER** to acknowledge the message, then answer Y to the **Clear method** prompt. Answering N here allows you to edit the method (see Section 5.6).

5.5.2 Entering breakpoints

A method consists of a series of breakpoints which define changes in one or more control parameters. Methods are programmed on a time or volume base, according to the current setting in Setup (see Section 5.2). The first breakpoint must be at time/volume 0.0. An alarm can be set anywhere in the method, e.g. to alert the operator for manual sample application or to signal the end of the method.

Note: If **FRACTN, VALVE ACTIVE** or **PEAK COLLECT** breakpoints are to be programmed within a gradient, then these breakpoints should be added after the gradient programming is completed. This avoids the need to calculate the exact value for **CONC %B** within the gradient. By programming as described above, GradiFrac will perform the calculation automatically.

Move through the program method menu with the arrow or **ENTER** keys, setting breakpoint parameters as required. If several breakpoints have been set, you can move up and down through the breakpoints, changing parameters as required.

5.5.3 Programming gradient shape

Set the concentration of buffer B with **CONC %B** (Figure 19).

Two breakpoints with different values for concentration of B will create a linear gradient from the first to the second value over the interval between the breakpoints. For example, the following breakpoints give a gradient from 25 to 45 %B, starting at 5 minutes and extending over 10 minutes:

TIME	**5.0 min**
CONC %B	**25**

TIME	**15.0 min**
CONC %B	**45**

Two gradient breakpoints separated by 0.1 ml or min will create a step gradient with an immediate change in buffer B concentration. For example, the following breakpoints cause an abrupt change from 25 to 45 %B at time 5 min.

TIME	**4.9 min**
CONC %B	**25**

TIME	**5.0 min**
CONC %B	**45**

5.5.4 Programming flow rate

Set the flow rate with **Flow ml/min** (Figure 19). A method cannot be programmed with zero flow rate.

Any changes in flow rate are made instantaneously at the breakpoint (time or volume) where the new value is requested.

5.5.5 Programming fraction size

Set the fraction size with **Fraction [min/ml/drop]** (Figure 19).

The fraction base (time, volume or drops) is determined by the current setting in Setup (see Section 5.2).

As long as the fraction size is set to 0, the flow diversion valve will divert effluent to waste.

5.5.6 Programming the auxiliary valve

Control the auxiliary valve where used with **Valve active [Y/N]** (Figure 19).

The auxiliary valve is connected to the **Aux** port. Enter Y to activate the valve and N to deactivate the valve. The current setting is indicated by Y or N in the instruction prompt.

5.5.7 Programming peak collection

Set peak collection with **Peak collect [Y/N]** (Figure 19).

Enter Y to activate peak collection. The fraction size must be set to >0 for peak collection to be activated. This may be done in the same breakpoint as that in which peak collection is activated.

If peak collection is activated, enter the threshold slope for peak detection as **Slope %/ml.** Values may be set from 1 to 99 %/ml. The fraction collector will change tubes whenever the slope exceeds the set value. The peak end is determined automatically by GradiFrac. The set fraction size applies both within and between peaks.

The following procedure is recommended for determining a suitable slope value:

1. Use a previous chromatogram from an identical run to determine the slope %/ml (see Figure 19). Peak collection is not recommended with an unknown sample.
2. Perform a blank run with the chosen setting to check that tube changes do not occur as a result of baseline disturbances. If unwanted tube changes occur, set a higher slope value and repeat the run.

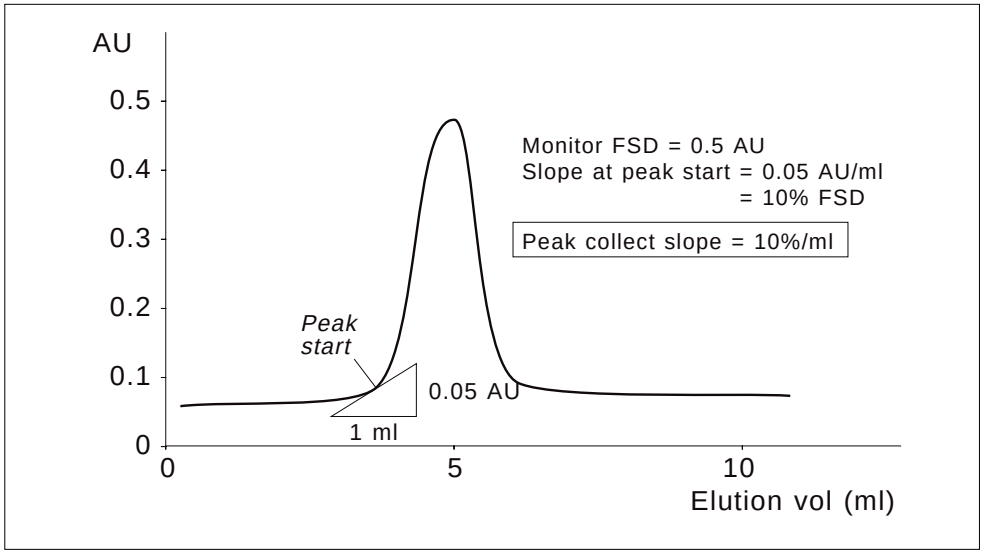


Fig. 20. Determining the slope value for peak collection (%/ml).

5.5.8 Ending the method

The method will end at the last breakpoint. If you require a period of constant parameters at the end of the method, enter a final breakpoint with the same parameters as the penultimate breakpoint (see the examples in Section 5.8).

When all breakpoints have been entered, press **end** to end method programming and answer Y to the **End method** prompt.

5.5.9 Programming an alarm

Set an alarm with **Alarm at** (Figure 19).

If you want an alarm to sound during or after the method, enter a non-zero value here. For example, entering **Alarm at** 1 min will sound an alarm 1 minute after the method is started.

5.6 Editing an existing method

To edit an existing method, choose Program method from the main menu. Select the number of the method and press enter.

GradiFrac responds with the message:

Method occupied!

Press **ENTER** to acknowledge the message, then answer N to the **Clear method** prompt. You may now edit the breakpoints in the existing method. Use the arrow keys at the **Edit breakpoint** prompt to scroll through the existing breakpoints, or enter the time or volume for an existing breakpoint to move directly to that breakpoint.

5.6.1 Inserting a breakpoint

To insert a breakpoint, enter the breakpoint time or volume at **Edit breakpoint** (Figure 19) and press **ENTER**. Set the parameter values as required. A newly entered breakpoint “inherits” default parameter settings from the closest previous breakpoint in the method.

5.6.2 Changing breakpoint parameters

To change the time or volume for a breakpoint, insert a new breakpoint at the required time or volume as described in Section 5.6.1, then delete the unwanted breakpoint as described in Section 5.6.3. You may need to edit parameter settings for the new breakpoint.

To change other parameters for an existing breakpoint, move to the required breakpoint, press **ENTER**, then change the parameters as required.

Remember that all parameters are set at every breakpoint. A new parameter value must be entered **for all breakpoints** where it is to apply. This is particularly important for flow rate and fraction size which are often set early in a method and apply throughout the method. If for example the flow rate is changed only at the first breakpoint, the previous flow rate will be restored at the second breakpoint.

5.6.3 Deleting a breakpoint

To remove an existing breakpoint, move to the required breakpoint and set the time/volume to 0 (use the **CE** key to clear the existing value). GradiFrac will ask you to confirm that the breakpoint should be deleted.

5.7 Documenting methods

The programmed gradient shape for any stored method may be documented on the recorder when method programming is completed. Set the recorder chart speed to 10 or 20 mm/s and answer Y to the prompt **SHOW CONC %B ON REC OUT** at the end of the Program Method block (Figure 19). The complete method is recorded in 10 s.

It is important that you make notes of the purpose and structure of methods stored in GradiFrac. Without these, it is easy to forget the purpose of a method which is identified by number alone, and it may be difficult to reconstruct the method if it is accidentally deleted.

The form on page 32 suggests a standard for recording method notes and breakpoints.

5.8 Method examples

Example 1 illustrates a simple GradiFrac method for a gradient elution with a linear gradient from 0 to 60 %B then an instantaneous change to 100 %B. Peak detection is set to on. The sample is applied manually after 1 minute, and fraction collection starts at the beginning of the gradient (allowing 25 ml flow-through to run to waste).

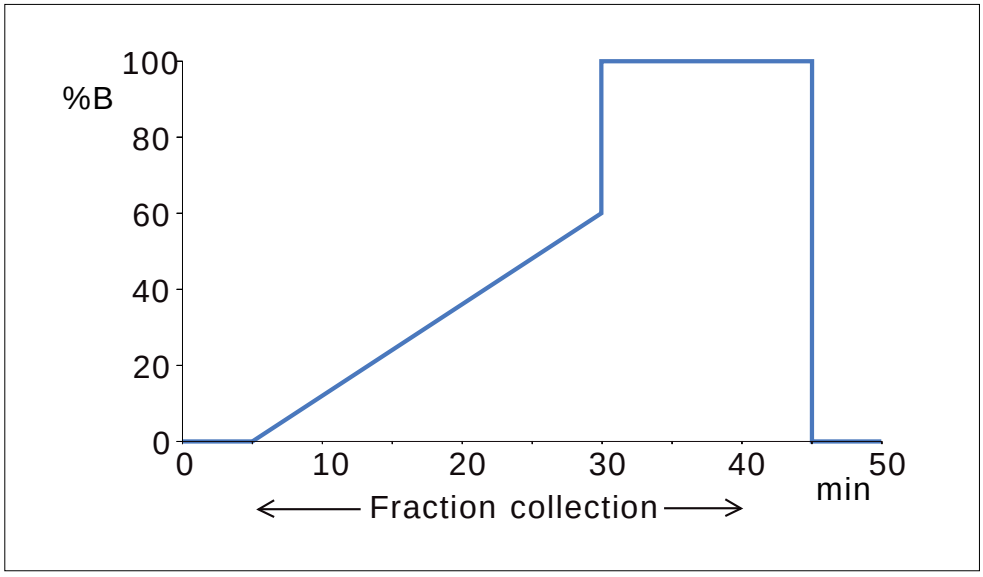


Fig. 21. Buffer gradient in the example method.

Table 5-1. Breakpoints for example method 1. Values actively entered for each breakpoint are shown in bold. Unchanged values are grey.

Time	Conc %B	Flow	Fractn	Valve	Peak	Slope	Comment
0.0	0	**5**	0	N	N	-	Start method
5.0	0	5	**0.5**	N	**Y**	**5**	Start gradient, start fractions
29.9	**60**	5	0.5	N	Y	5	End gradient
30.0	**100**	5	0.5	N	Y	5	Start column wash
44.9	100	5	0.5	N	Y	5	End column wash
45.0	**0**	5	**0**	N	**N**	-	End fractions start re-equilibration
50	0	5	0	N	N	-	End method
Alarm at 1 min							Alert for sample application

Note the final breakpoint at 50 minutes which ensures that the method continues for 5 minutes after the concentration of B is reduced to 0.

Example 2 shows illustrates a method with the same gradient as above, but using an auxiliary valve for sample application (see system configuration in Appendix). In example 2, fractions are collected between 10 and 20 minutes, starting and stopping within the gradient. Note how these breakpoints are entered after the gradient is programmed, allowing GradiFrac to calculate the value for CONC %B.

Table 5-2. Breakpoints for example method 2. Values actively entered for each breakpoint are shown in **bold**. Unchanged values are grey.

Time	Conc %B	Flow	Fractn	Valve	Peak	Slope	Comment
0.0	0	**5**	0	N	N	-	Start method
1.0	0	5	0	**Y**	N	-	Start sample application
2.0	0	5	0	**N**	N	-	End sample application
5.0	0	5	0	N	N	-	Start gradient
29.9	**60**	5	0	N	N	-	End gradient
30.0	**100**	5	0	N	N	-	Start column wash
44.9	100	5	0	N	N	-	End column wash
45.0	**0**	5	0	N	N	-	Start re-equilibration
50.0	0	5	0	N	N	-	End method
10.0	12	5	**0.5**	N	**Y**	**5**	Start fractions
20.0	36	5	**0**	N	**N**	-	End fractions
Alarm at 50 min							Signal method end

Method no:..... Author:..... Date:.....

Column type: ..... Auxiliary valve used?

Setup parameters:

Method base: time ❑ volume ❑ Fractionate by: time ❑ volume ❑ drop ❑

Pump calib. value: ..... Delay UV to frac: .....

Mix vol:..... Drop sync active: .....

Notes:

Breakpoints:

Time/volume	Conc %B	Flow	Fractn	Valve	Peak	Slope	Comment

6. Operation

6.1 Manual operation

To control GradiFrac manually without using a preprogrammed method, choose Manual Run in the main menu and press **ENTER**.

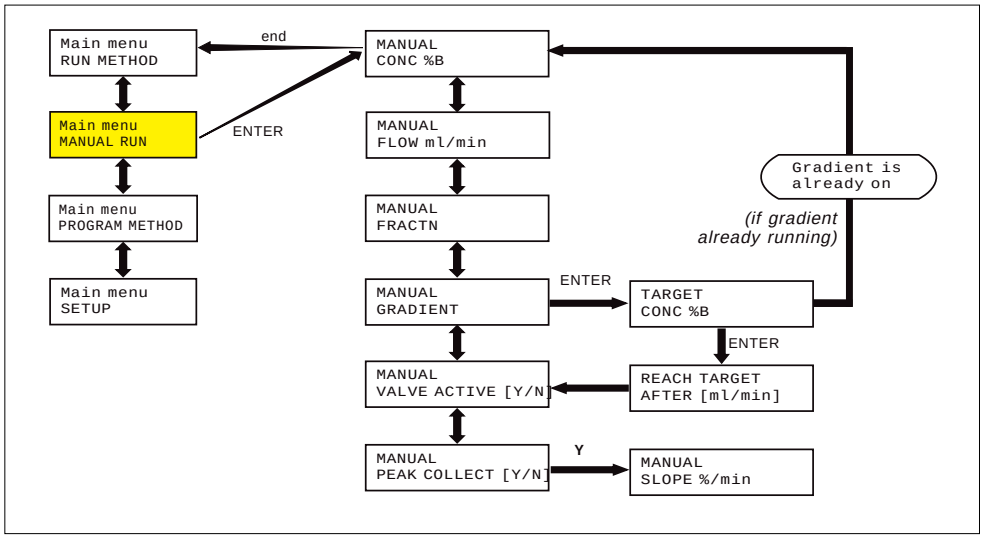


Fig. 22. The Manual Run control block.

Use the arrow keys to move through the menu options, setting parameters as required. Settings take effect as soon as the instruction is confirmed with **ENTER**. You can change parameters at any time during manual control, with the exception of the gradient. Gradient parameters can only be changed

1) if there is no gradient running, or
2) if the system is paused or held.

To end manual control, press **end**.

6.1.1 Setting a gradient

Set a constant concentration of buffer B with **CONC %B** (Figure 22). The concentration will apply as soon as you press **ENTER**. To change the concentration instantaneously (step gradient), simply enter a new value.

To set a gradient, move to **GRADIENT** (Figure 22), press **ENTER**, then enter the target concentration of buffer B and the time or volume after which the target is to be reached. The base is determined by the current method base setting in Setup (see Section 5.2). For example, the following entries start a gradient from the current concentration to 70% B over a period of 10 minutes:

TARGET	
CONC %B	**70**

```
REACH TARGET
AFTER min        10.0
```

If a gradient is already running, GradiFrac will respond with the message

```
Gradient is
        already on
```

The display changes to **CONC %B** after 3 s. Press **pause** or **hold** followed by **ENTER** to stop the gradient and enter new parameters.

A gradient can only be started if the pump is running. If a gradient is requested when no flow has been set, GradiFrac will respond with the message

```
No Flow
```

and return to the beginning of the manual run menu.

The display indicates the current gradient direction with the symbols:

/ increasing % B

– constant (isocratic) % B

\ decreasing % B

6.1.2 Setting flow rate

Set the flow rate with **Flow [ml/min]** (Figure 22). The pump will start (or change speed if already started) when you press **ENTER**.

6.1.3 Setting fraction size

Set the fraction size with **Fraction [min/ml/drop]** (Figure 22). The fraction base (time, volume or drops) is determined by the current setting in Setup (see Section 5.2). The fraction size will apply as soon as you press **ENTER**.

As long as the fraction size is set to 0, the flow diversion valve will divert effluent to waste.

6.1.4 Operating the auxiliary valve

Operate the auxiliary valve where used with **Valve active [Y/N]** (Figure 22).

The auxiliary valve is connected to the **Aux** port. Enter Y to activate the valve and N to deactivate the valve. The current setting is indicated by Y or N in the instruction prompt.

6.1.5 Setting peak collection

Set peak collection with **Peak collect [Y/N]** (Figure 22).

Enter Y to activate peak collection. The peak collection function is explained on p. 28.

6.2 Method-controlled operation

This section describes the use of GradiFrac for method-controlled runs. It is assumed that GradiFrac has been installed as described in Chapter 4 and that at least one method has been programmed. Method programming is described in Section 5.4.

6.2.1 Running a method

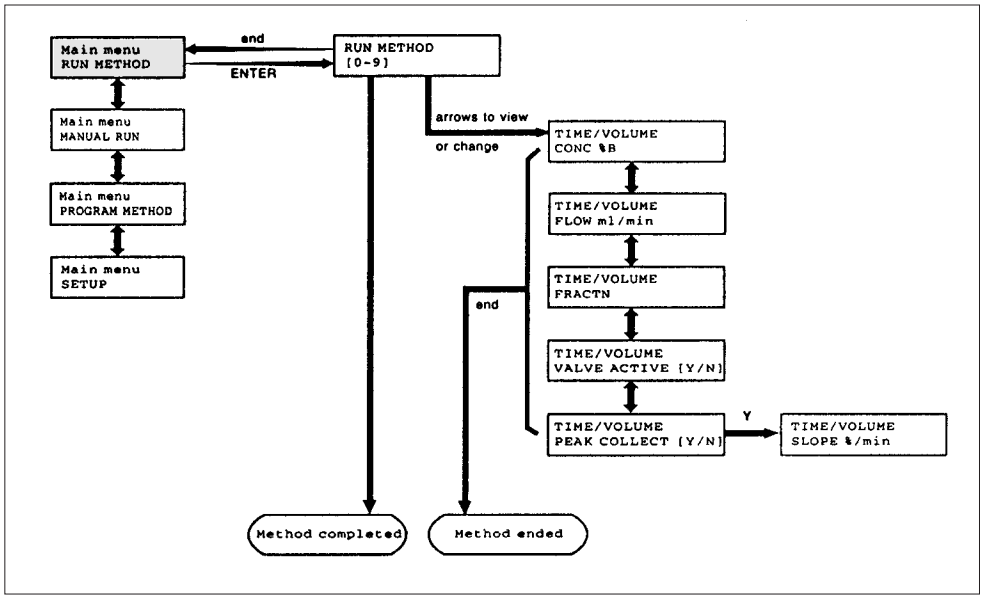


Fig. 23. The Run method control block.

1. Fill the tube rack with tubes, starting at position 1, and adjust the delivery arm so that the tube sensor rests against the outer side of tube no. 1.
2. Choose Run method from the main menu and press **ENTER**:
3. Enter the number of the method to run (0-9: the numbers of programmed methods are displayed) and press **ENTER** to start the method. If you enter the number of a method which has not been programmed, GradiFrac will respond with the message **Method is empty**. Press **ENTER** to acknowledge and select another method number.
4. When the method is finished, GradiFrac will display the message **Method is completed**. Press **end** to return to the main menu. Press **ENTER** twice to re-run the method.

6.2.2 Interrupting a method

There are three ways to interrupt a method:

pause stops method operation but does not end the method. Press **continue** to resume method operation.

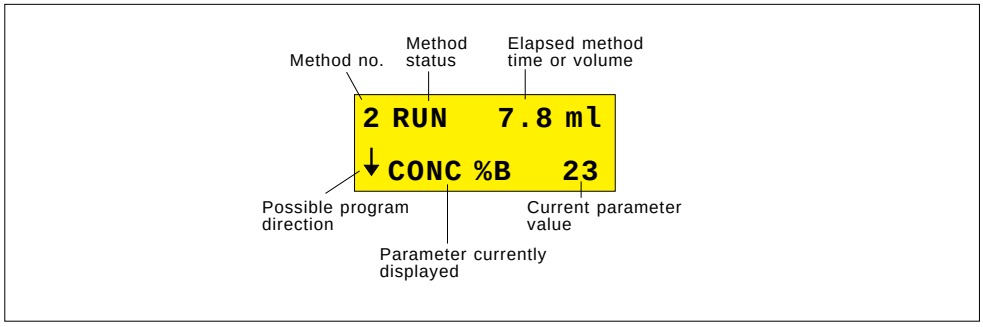
hold holds method time/volume and stops gradient changes but continues to operate the pump and fraction collector. Press **hold** again to display the accumulated volume. Press **continue** to resume gradient formation.

end interrupts the method with the prompt **End method Y/N**. Entering **Y** terminates the method. Entering **N** resumes method operation.

6.2.3 Examining current method status

During method operation, the arrow keys can be used to scroll through display of the current method status.

The following information is displayed:



6.2.4 Manual control during method operation

The parameter values in method status display may be replaced with new values to modify method operation.

A parameter which is changed manually continues to apply until it is reset by a breakpoint with a value different from that originally programmed in the method. For example, consider a method with breakpoints at 0, 5 and 10 minutes with flow rate 2, 2 and 4 ml/min respectively:

0.00 FLOW 2.0 ml/min

5.00 FLOW 2.0 ml/min

10.00 FLOW 4.0 ml/min

If the flow rate is changed manually to 3 ml/min after 2 min, this value will continue to apply until the breakpoint at 10 min.

Gradients can only be changed in Hold or Pause.

7. Maintenance

GradiFrac should be cleaned thoroughly after use. Inadvertently spilled liquid should not be allowed to dry on the instrument and the delivery arm should always be returned to its central position over the bowl when not in use.

No regular maintenance is required.

The surfaces of GradiFrac may be wiped with mild aqueous cleaning solutions or with ethanol.

Maintenance of the power supply should be carried out by authorised Amersham Biosciences personnel only.

Maintenance of other components in GradiFrac System is described in the respective manuals.

8. Troubleshooting

The table below lists messages which may appear on the GradiFrac display, with explanations and suggested actions.

If other error messages appear for which the corrective action is not obvious, contact technical service.

Message	Cause	Action
Tube pos error	Tube sensor active due to jammed rack.	Check for obstructions to rack movement and remove them.
	Tube sensor broken	Contact Amersham Biosciences service engineer.
No more tube available	No more tubes.	Fill rack with more tubes.
	Rack movement jammed	Check for obstructions to rack movement and remove them.
	Delivery arm in stand-by position.	Position the delivery arm correctly.
Method is empty!	Attempt to run method without instructions	Choose a different method number.
Warn: Check that tubepos is OK	Tube sensor between tubes (only at start of run).	Position the GradiFrac bowl so that a tube is directly under the flow outlet.
No Flow	Attempt to start a manual gradient or program a breakpoint with no flow.	Set a flow rate manually or as the first breakpoint in the method.
Stop gradient in HOLD or PAUSE	Attempt to change CONC %B during a running gradient.	Press **hold** or **pause** and stop the gradient first.
Recalibrate pump or flow too high	Too high flow rate or incorrect calibration factor	Set a lower flow rate or recalibrate the pump.
Recalibrate pump or flow too low	Too low flow rate or incorrect calibration factor	Set a higher flow rate or recalibrate the pump.
Warning: Switch valve too fast	Mix volume too low for requested CONC %B at this flow rate.	Set a higher mix volume. (The warning may be ignored at CONC %B close to 0 or 100 in a gradient).
Switch cycle time too fast	Flow rate too high or mix volume too low.	Set a lower flow rate or higher mix volume.

Message	Cause	Action
Gradient is already on	Attempt to change CONC %B during a running gradient (manual mode).	Wait until the gradient is complete, or press **pause** or **hold** followed by **ENTER** to stop the gradient.
Warning: Pump is not operable	Attempt to start pump failed.	Check that the pump is switched ON and that the communication cable is properly connected. Press **ENTER** then **continue** to ignore the message (pump must be controlled separately).
Pump OverPresssure	The pressure limit for the pump has been reached (HiLoad Pump P-50 only).	Find and correct the cause of the overpressure. Tubing or column may be blocked, or the flow rate may be too high for the column.
Pump RUN is not pressed	The RUN button on the pump is not pressed (HiLoad Pump P-50 only).	Press the RUN button on the pump.
Too short time between feeds	Flow rate too high or fraction size too small.	Reduce the flow rate or set a larger fraction size.
No fraction size	Attempt to use peak collection with no fraction size.	Set a fraction size before activating peak collection.
No drops, check sensor	No drops detected (drop sync mode or drop fraction base only).	Check that buffer drops from the outlet. Clean the drop sensor. If the error persists, contact Amersham Biosciences service or run without drop sync and drop counting.
Continuous flow, check sensor	No drops detected due to too high flow rate or dirty drop sensor.	Reduce the flow rate or clean the drop sensor. If the error persists, contact Amersham Biosciences service or run without drop sync and drop counting.
Method memory is full	Attempt to program more than 52 breakpoints in a method.	Reduce the number of breakpoints in the method.
No alarm after end of method	Attempt to set an alarm later than method completion.	Set the alarm within the method. (Use extra breakpoints with zero flow rate to extend the method time).
Bad drop sensor or not connected	Drop sensor cable not connected or dirty drop sensor (setup mode only).	Check the drop sensor connection or clean the drop sensor.
Endpoint missing, edit your method	The method consists of only one breakpoint.	Add breakpoints to the method.

9. Technical specifications

GradiFrac

Programming base	ml or min
Programming capacity	10 separate methods, 52 breakpoints each
Memory protection	EEPROM
Pump control	Remote for Pump P-1 and HiLoad Pump P-50
Gradient formation	Single pump and switch valve

Fraction collection

Time base	0.1–99.9 min
Volume base	0.1–99.9 ml
Drop base	1–999 drops
Tube change time	0.3–0.6 s (tube rack 18 mm)
Rack capacity	95 tubes, rack 18 mm
Event mark	50 ms contact closure
Flow diverter	Solenoid valve PSV-50
Monitor input range	0–10 mV, 0–100 mV, 0–1000 mV
Recorder control	Controls REC 101 and REC 102
Valve control	3 outports for Solenoid Valve PSV-50 (switch valve, flow diverter, auxiliary)
Mixer	0.6 ml
Power supply	24 V DC Power Supply for 100–240 VAC, 50-60 Hz
Power consumption	15 VA maximum
Dimensions (w x d x h)	290 x 300 x 390 mm
Weight	3.9 kg
Environment	4 to 40 °C, 20-95% relative humidity, 84-106 kPa (840-1060 mbar) atmospheric pressure

Safety standards

EMC

This product meets the requirement of the EMC Directive 89/336/EEC through the harmonized standards EN 50081-2 (emission) and EN 50082-1 (immunity)

Note: This is a class A product. In a domestic environment this product may cause radio interference in which case the user may be required to take adequate actions.

Note: The declaration of conformity is valid for the instrument when it is :

- used in laboratory locations
- used in the same state as it was delivered from Amersham Biosciences AB except for alteration described in the User Manual
- used as ”stand alone” unit or connected to other CE labelled Amersham Biosciences products or other products as recommended

Safety

This product meets the requirement of the Low Voltage Directive (LVD) 73/23/EEC through the harmonized standard EN 61010-1.

10. Accessories

Please order accessories according to the designation and code numbers given below. Refer to Figure 24 for position number where given.

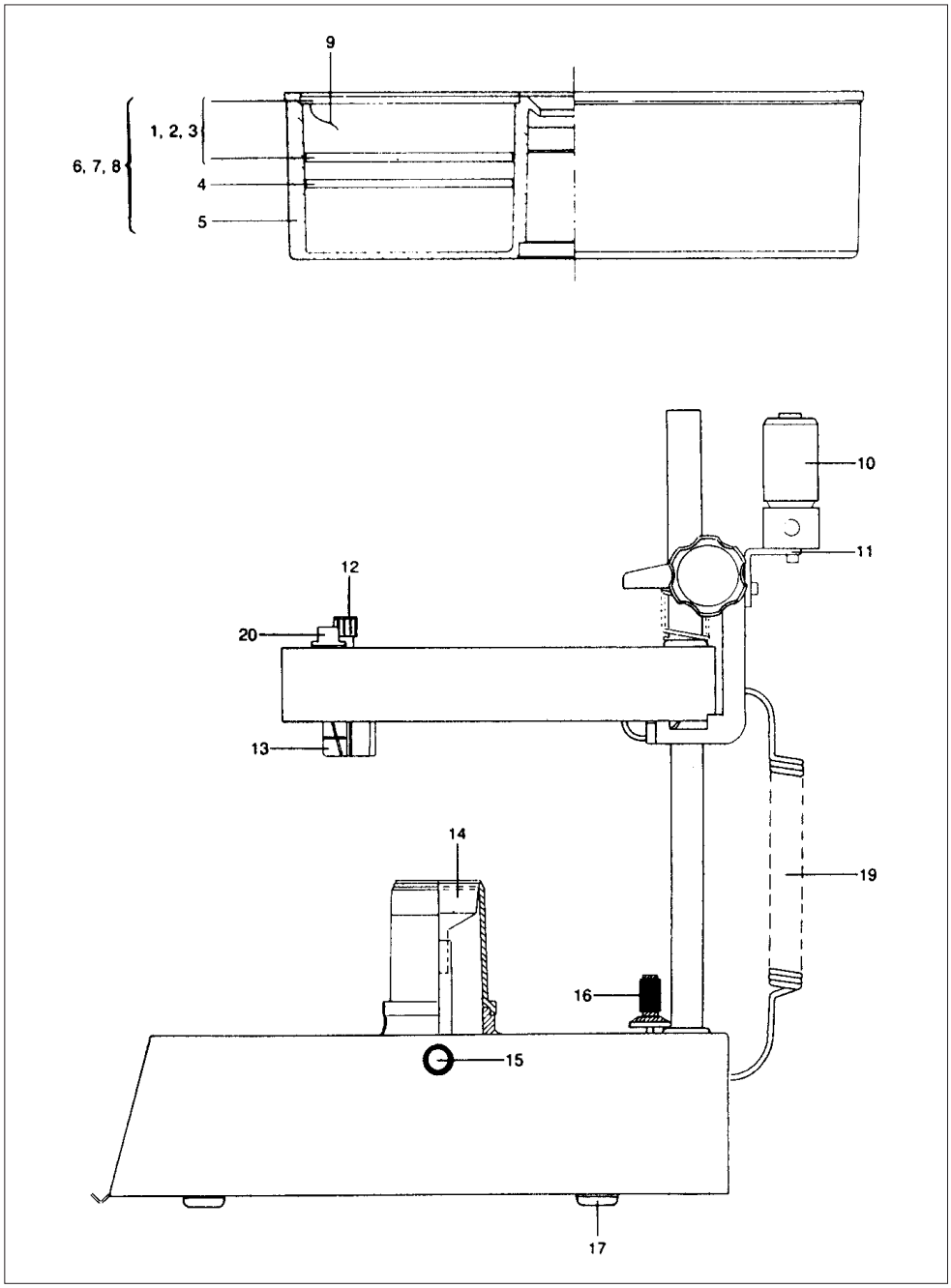


Fig. 24. GradiFrac accessories.

11. Ordering information

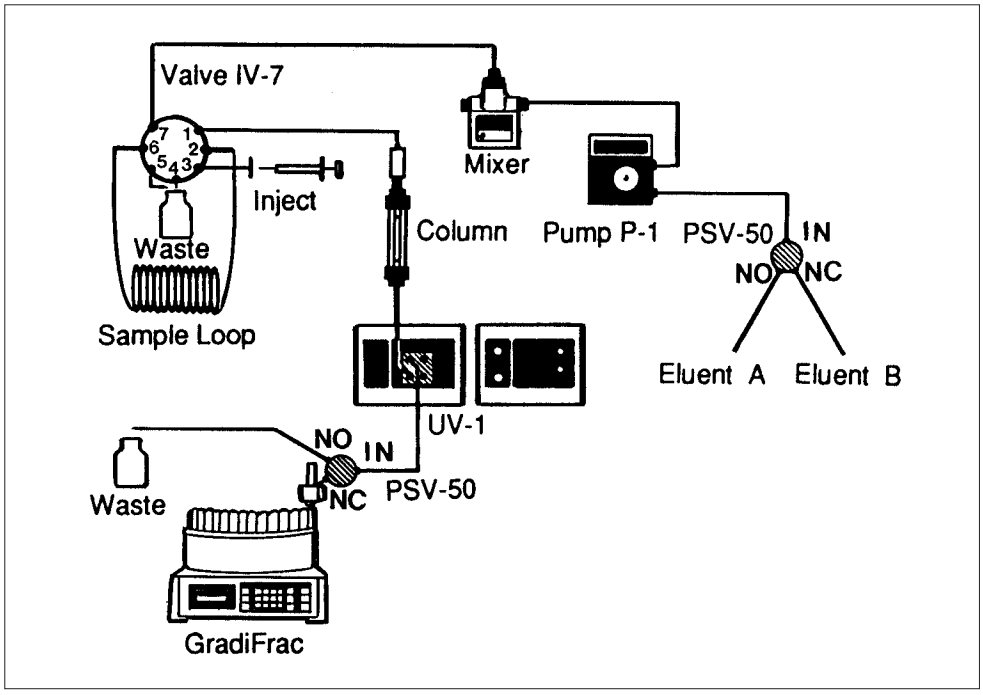
Designation		Code No.
GradiFrac System components		
Peristaltic Pump P-1		18-1110-91
Monitor UV-1, Control and Optical Unit		18-1003-66
Flow cell 10 mm		19-2504-02
280 nm Filter Kit		19-2433-01
Recorder REC 102, 2-channel		18-1001-43
Mixer 0.6 ml		19-6700-01
Injection Valve IV-7		19-1996-01
Sample loops, 1 and 2 ml		18-5897-01
Rack		18-1993-05
Valve Bracket Kit		19-1994-50
Tubing connector/M6 male, pkg/5		19-1993-50
Tubing ETFE, 0.8 mm i.d., 1.8 mm o.d., 2 m		19-7435-01
Valve Holder Kit		19-1994-70
Recorder Cable		18-1107-88
Power supply 24 V		18-1032-06
Optional		
HiLoad Pump P-50		19-1992-01
Mixer 6 ml 110 V		18-3500-01
Mixer 6 ml 220 V		18-3501-01
Solenoid valve PSV-50		19-1994-01
Accessories (refer to Figure 10-1)	Position no.	
Tube holder/guide, 12 mm	1	19-7242-02
Tube holder/guide, 18 mm	2	19-8689-02
Tube holder/guide, 30 mm	3	18-1124-68
Tube support	4	18-3054-02
Bowl	5	18-3051-03
Tube rack complete, 12 mm	6	19-8684-03
Tube rack complete, 18 mm	7	18-3050-03
Tube rack complete, 30 mm	8	18-1124-67
Tube spring, metal (for 18 mm holder/guide)	9	19-6057-01
Valve PSV-50	10	19-1994-01
Valve holder kit	11	19-6069-01
Tubing holder	12	18-6464-01
Tube sensor kit	13	19-7815-02
Funnel	14	19-6044-01
Funnel tubing 12/8, 250 mm	15	18-3020-01
Drive sleeve	16	19-6067-02
Spiral cable	19	19-8638-01
Sensor positioning kit	20	18-6470-01
Accessories available but not illustrated		
Drop Counter Kit		18-1012-05
Communication cable, Pump P-1		19-6005-02
Signal cable, UV-1		18-0577-01
Tube holder kit 8/11 mm		18-8522-01

Designation	Code No.
Green connector 4-pole female	18-0863-01
Green connector 2-pole female	19-8629-01
254 nm Filter Kit	19-2432-01
405 nm Filter Kit	19-4724-01
Inlet tubing	19-4448-01
Assorted sample loops (25, 50, 100, 200, 500 ml)	18-0404-01
SuperloopTM 10 ml (together with Pump P-50)	19-7585-01
SuperloopTM 50 ml (together with Pump P-50)	19-7850-01
Clamp holder, pkg/5	18-1032-03
Gel filtration columns	
HiLoad 16/60 Superdex 75 prep grade	17-1068-01
HiLoad 26/60 Superdex 75 prep grade*	17-1070-01
HiLoad 16/60 Superdex 200 prep grade	17-1069-01
HiLoad 26/60 Superdex 200 prep grade*	17-1071-01
HiLoad 16/60 Sephacryl S-100 HR	17-1165-01
HiLoad 26/60 Sephacryl S-100 HR	17-1194-01
HiLoad 16/60 Sephacryl S-200 HR	17-1166-01
HiLoad 26/60 Sephacryl S-200 HR	17-1195-01
HiLoad 16/60 Sephacryl S-300 HR	17-1167-01
HiLoad 26/60 Sephacryl S-300 HR	17-1196-01
Ion exchange columns	
HiLoad 16/10 Q Sepharose HP	17-1064-01
HiLoad 26/10 Q Sepharose HP*	17-1066-01
HiLoad 16/10 S Sepharose HP	17-1065-01
HiLoad 26/10 S Sepharose HP*	17-1067-01
HiLoad 16/10 Q Sepharose FF	17-1060-01
HiLoad 26/10 Q Sepharose FF*	17-1062-01
HiLoad 16/10 S Sepharose FF	17-1061-01
HiLoad 26/10 S Sepharose FF*	17-1063-01
RESOURCE Q 1 ml	17-1177-01
RESOURCE Q 6 ml	17-1179-01
RESOURCE S 1 ml	17-1178-01
RESOURCE S 6 ml	17-1180-01
Hydrophobic interaction columns	
HiLoad 16/10 Phenyl Sepharose HP	17-1085-01
HiLoad 26/10 Phenyl Sepharose HP*	17-1086-01

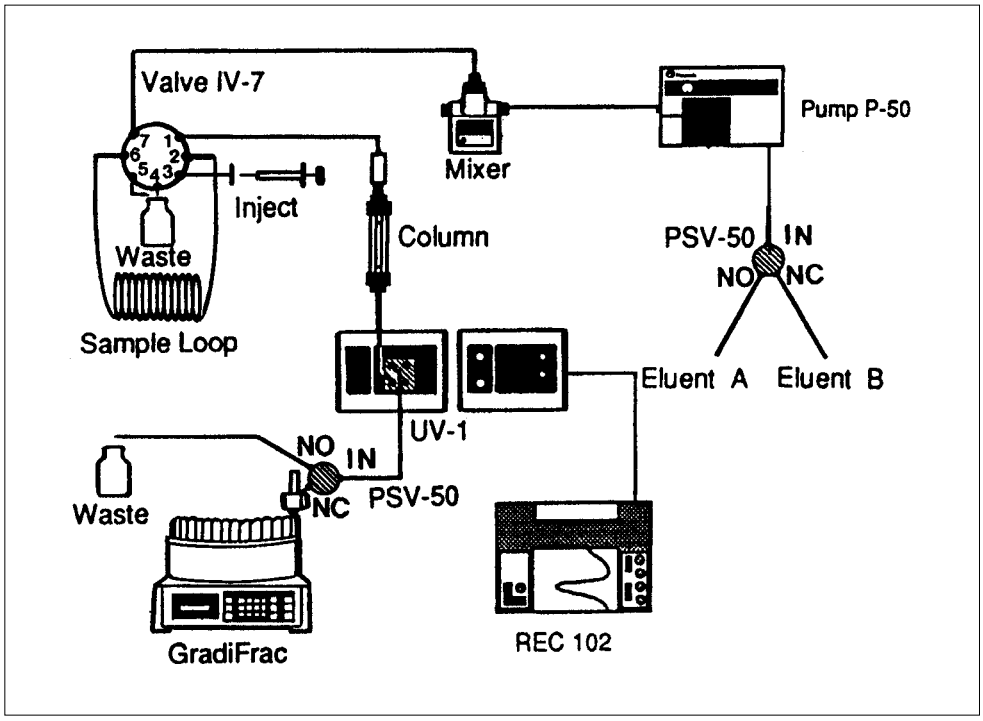
*HiLoad pump P-50 is recommended for best performance with these columns.

Affinity chromatography columns	
HiTrap Protein A, 5x1 ml	17-0402-01
HiTrap Protein A, 1x5 ml	17-0403-01
HiTrap Protein G, 5x1 ml	17-0404-01
HiTrap Protein A, 1x5 ml	17-0405-01
HiTrap Heparin, 5x1 ml	17-0406-01
HiTrap Heparin, 1x5 ml	17-0407-01
HiTrap Chelating, 5x1 ml	17-0408-01
HiTrap Chelating, 1x5 ml	17-0409-01
HiTrap Blue, 5x1 ml	17-0412-01
HiTrap Blue, 1x5 ml	17-0413-01
HiTrap NHS-activated, 5x1 ml	17-0416-01
HiTrap NHS-activated, 1x5 ml	17-0417-01

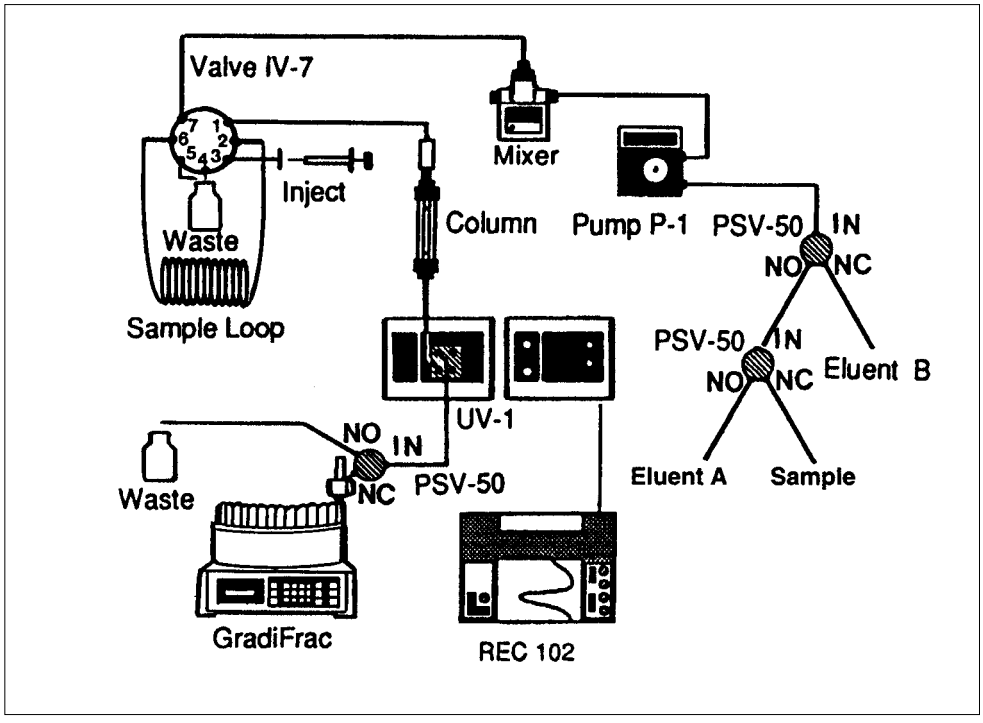
Appendix
System configurations



GradiFrac System, two-valve configuration with Pump P-1.



GradiFrac System, two-valve configuration with HiLoad Pump P-50.



GradiFrac System, with auxiliary valve for sample application.

Index

A

accessories
- connection 5,18
- ordering information 42

adjusting the delivery arm 16
alarm....29
arrow keys 4
assembling tube racks
- 12 mm racks....13
- 18 mm racks....13
- 30 mm racks....15

automatic sample application....8, 10
auxiliary valve....8, 10
- connection 19
- manual control 34
- mounting....17
- programming....28

B

bowl mounting....15
breakpoints 26
- changing parameters....29
- deleting....29
- inserting....29

C

calibrating pumps....25
cleaning....37
connecting accessories 5
connections
- electrical 18
- tubing....20

continue key....5, 35
custom tube racks 6

D

delay UV to frac 24
delivery arm 7
delivery tubing
- fitting....16
- recommended 8

display
- method status 36

display messages....38
display window 4
documenting methods 30
drop counter 7
drop synchronization 25

E

editing a method 29
electrical connections 18
end key....4, 35
ENTER key....4
event mark 11
example methods 30

F

feed tube key....4
fitting delivery tubing 16
flow diversion....28
flow diversion valve....7, 10
- connection 19
- mounting....16

flow rate
- manual setting 34
- programming....27

flow rate range 9
fraction size
- manual setting 34
- programming....28

fractionation base....22, 23, 28
front panel 4
function keys....4

G
gradient valve 7, 10
connection 19
mounting 17
gradients
manual alterations 34
manual control 33
programming 27
GradiFrac System 9
configuration 44
mounting 17

H
HiLoad Pump P-50 9
hold key 5, 35

I
injection valve 10
interrupting a method 35

K
keyboard 4

L
linear gradient
manual 33
programming 27

M
main menu 22
maintenance 37
manual auxiliary valve control 34
manual flow rate setting 34
manual fraction size setting 34
manual gradients 33
manual operation 33
manual peak collection 34
manual run menu 33
method base 22, 23
method number
selecting for programming 26
methods
documenting 30
examples 30
interrupting 35
manual intervention 35
programming 26
running methods 35
status 36
mix volume 24
mixer 10
connection 19
monitor 11
connection 11, 20
monitor range 6, 11, 20
monitor-to-collector delay 24
mounting the bowl 15
mounting valves 16

N
numerical keys 4

P
pause key 4, 35
peak collection
manual control 34
programming 28
threshold slope 28
power conversion unit 6
power supply 6
connection 20
power switch 5
program method menu 26
programming an alarm 29
programming auxiliary valve 28
programming flow rate 27
programming fraction size 28
programming gradients 27

programming peak collection 28
pump calibration
factor 24
procedure 24
pump calibration factor
for HiLoad Pump P-50 26
pump connector 9
Pump P-1 9
pumps
connection 19
control 9
recommended 9

R

rack 9
component placing 18
rear panel 5
recorder 10
chart speed 20
connection 20
recorder start/stop 10
run method menu 35
running a method 35

S

sample application valve 10
setup menu 22
setup parameters 22
slope for peak collection 28
solenoid valves 7, 10
connection 19
mounting 16, 17
outlets 7
tubing connections 20
specifications
GradiFrac 40
step gradient
manual 33
programming 27
system components 9
system configuration 44
system shut-down 21
system start 21

T

three-buffer systems 7, 10
troubleshooting 38
tube racks 6
assembly 12
components 12
tube sensor 6
connection 19
tube sensor switch 7
tubing connections 20
tubing holder 7
tubing length guide 7, 16

V

valves
see solenoid valves

W

waste collector 7

Y

Y/N key 4

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